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Contents of previous issues of the JOURNAL OF ANIMAL SCIENCE are indexed in the Agricultural Index which is available in most libraries and in the November issue of each complete volume of the JOURNAL.

A COMPARISON OF THE LIGNIN RATIO TECHNIQUE AND THE CHROMOGEN METHOD OF DETERMINING DIGESTIBILITY AND FORAGE CONSUMPTION OF DESERT RANGE PLANTS BY SHEEP[1]

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THE nutritive value of range forage has become a subject of much interest during the past few years. However, the problem has received little study and as a result both livestock and range managers are groping for practical information from only a meager amount of scientific data. Most studies dealing with the nutrients supplied from native range plants have dealt primarily with chemical analyses of bulk samples of herbage collected from the range. Nutritive value as measured by chemical content alone is not a reliable index to availability of the various nutrients contained in the forage unless accompanied by digestibility determinations or balance trials. With such information, available nutrients can be compared to recommended standards and accurate appraisals of the nutritive value of various range plants can be made.

It has long been recognized that digestion coefficients could be determined for animals grazing on the range if a "tracer" plant constituent could be found. This tracer constituent should appear normally in the plant and be indigestible so that it may be recovered in the feces. Animals can then be equipped with fecal bags and allowed to select their forage from the range in a normal manner. For practical purposes lignin apparently meets this requirement (Ellis *et al.*, 1946; Forbes *et al.*, 1946; and Forbes and Garrigus, 1948). Some controversy has arisen over the validity of lignin being an indigestible plant constituent (Bondi and Meyer, 1948; Csonka *et al.*, 1929; and Davis *et al.*, 1947). However, Chi (1951), Ellis *et al.* (1946), Forbes *et al.* (1946), and Forbes and Garrigus (1948) show that digestion coefficients for lignin fluctuate slightly above and below zero and the average approaches zero. Therefore it appears that digestion coefficients calculated by the lignin ratio technique would be sufficiently accurate

[1] This research was supported in part by a grant from the International Minerals and Chemical Corporation, Chicago, Illinois and from the American Dehydrators Association, Chicago, Illinois.

[2] Associate Research Professor of Range Management, and Chairman, Institute of Nutrition, respectively.

[3] Logan, Utah.

for practical field application, since they vary from time to time due to soil, site, climatic conditions, stage of maturity and plant species.

Recently Reid *et al.* (1950) reported that plant pigments might serve as tracer or indicator substances suitable for determining digestibility by indirect methods. This procedure was introduced as the chromogen method which employed plant pigments that absorb light at 406 mμ as the reference or tracer substance.

Since it was stated by the joint committee report on pasture and range research technique (1950) that the chromogen method would soon replace the lignin ratio technique, it was deemed advisable to compare the two methods under actual range conditions.

Procedure

This study was conducted during the winter grazing season of 1950–51 on desert ranges near Kelton in northwestern Utah. This desert range area is typical of the northern desert shrub vegetation. The forage cover is composed of complex mixtures of plant species with local areas completely dominated by single species. The major forage species found throughout the area in pure stands as well as in mixed stands are big sagebrush *(Artemisia tridentata)*, shadscale *(Atriplex confertifolia)*, winterfat *(Eurotia lanata)*, black sage *(Artemisia nova)*, and squirreltail grass *(Sitanion hystrix)*. These are all persistent leaved browse or shrubby species with the exception of squirreltail grass.

Big sagebrush is found on foothill and desert areas throughout the Great Basin region where the soils are relatively alkali free. Where the desert soils contain even moderate amounts of alkali the area is dominated by shadscale and other salt tolerant species. Shadscale is a comparatively small shrub with spiney stems and produces an abundance of seed that is readily eaten by sheep early in the season. Winterfat is often called a half shrub because it possesses only a woody base. The remainder of the plant is herbaceous and is covered with a fuzzy material. Black sage closely resembles big sagebrush but is generally smaller and confined to rocky or gravelly soils.

Squirreltail grass during this study resembled most mature grasses that have been exposed to weathering for several months.

All species were in a dormant stage and did not display characteristics of lush growing vegetation.

Seven wether sheep, from 3 to 4 years of age, were grazed on vegetation types composed of a single species. These animals were equipped with specially constructed fecal bags and allowed to graze

in temporary enclosures in a normal manner (fig. 1). The bags were emptied twice daily and composite samples for a six-day grazing period were taken to the laboratory for analysis. The wethers were allowed an eight-day preliminary feeding period before collection of feces was started.

Forage samples were taken by observing individual animals and hand plucking forage comparable to the material actually being consumed by the sheep. Many small random plucks were taken over the area with the sheep as they were normally grazing. The individual collecting the forage samples walked along among the sheep while they were grazing during the day and plucked only the portion of the plant being utilized by the foraging animals. Samples were taken one day previous to the time feces collections were to be started and continued each day until the termination of the 6-day collection period.

Figure 1. Showing wether sheep equipped with fecal bags while grazing a temporary enclosure on big sagebrush forage.

Forage and feces samples were handled in the same manner. They were never exposed to light or temperatures above 30 degrees centigrade before being transported to the laboratory for chemical analysis.

Lignin was determined by the method of Ellis *et al.* (1946) and chromogen by the method of Reid *et al.* (1950). Both of these methods were modified as suggested by the authors through personal correspondence.

In the present study it was found that freezing, drying, or grinding had no effect upon the chromogen content for brief periods of one to two weeks as long as the material was not exposed to light.

TABLE 1. A COMPARISON OF THE LIGNIN AND CHROMOGEN CONTENT IN THE FECES OF SIX SHEEP FROM TWO FORAGE SOURCES

Sheep	Big sagebrush		Shadscale	
	Lignin content	Chromogen content	Lignin content	Chromogen content
	percent	units/gram	percent	units/gram
1	23.4	58	25.0	109
2	24.6	44	23.9	113
3	26.2	50	22.7	96
4	22.3	50	23.7	102
5	24.2	52	24.7	90
6	24.0	56	25.0	112
Average	24.1	52	24.2	104
Standard deviation	2.9	11.1	2.0	20.8
Coefficient of variation	11.9	21.3	8.3	20.0

Results and Discussion

It was found that chemical analyses of the lignin and chromogen content of range forage could be determined with equal accuracy. The differences between duplicate samples and duplicate determinations of the same sample were insignificant at the one percent level in all cases. However, when differences among animals were compared by the two methods for various species, the lignin technique gave considerably less variability compared to the chromogen method (table 1).

The coefficient of variation for lignin content of feces among animals grazing big sagebrush was 11.9 percent compared to 21.3 percent for chromogen content. A similar relationship was found when comparing the lignin and chromogen content of feces of animals grazing shadscale. The coefficients of variation in this comparison were 8.3 percent for lignin and 20.0 percent for chromogen.

The amount of chromogen recovered in the feces while sheep grazed big sagebrush and black sage was markedly less than the amount

consumed, showing that some of the chromogen was lost or was changed during digestion to give different light absorption values. This of course gave negative digestion coefficients and indicated extremely low quantities of consumed forage (table 2). However, in the case of squirreltail grass the chromogen recovery in the feces seemed high and gave a coefficient of digestibility for dry matter of 68.7 percent which is excessively high for mature dry grass that has been exposed to weathering for a considerable length of time. In addition, calculated dry matter intake was almost 7 pounds per 142-pound wether which is beyond the amount generally accepted for this size sheep. The lignin ratio technique agrees rather closely with calculated dry matter intake as presented by the National Research Council (1949) and digestibility of dry matter of similar forages presented by Schneider (1947).

Both methods agree closely in results from feeding good quality alfalfa hay. Digestibility of dry matter as calculated from the lignin ratio technique was 61.9 percent compared to 60.7 percent for the chromogen method (table 2). However, on most range forages there was wide variability and this was greater in the case of the two sagebrush species. These two species had a high content of ether extract, being 17.5 percent for big sagebrush and 11.1 percent for black sage. Other range forage species contained less than 3 percent. These two sagebrush species are rather high in essential oils which are readily absorbed in the process of digestion (Guenther, 1948 and 1949). It is believed that these essential oils might feasibly carry some of the plant pigments through the intestinal wall and the chromogen would be eliminated with the metabolized products of the essential oils in the urine. Analysis of urine from animals consuming black sage compared to urine analysis from animals consuming squirreltail grass showed that the chromogen content of the urine from animals on black sage was about 16 times more compared to urine taken from animals grazing grass. This does not necessarily confirm the theory that the plant pigments in question are absorbed and eliminated in the urine but it does suggest the possibility that such could be the case.

Absorption curves of acetone extracts at various wave lengths for forage and feces of range plants when adjusted to intersect at a wave length absorption at 406 mμ showed that the curves did not parallel each other as they did for alfalfa hay (fig. 2). This gives considerable error in the ratio of chromogen content in the feces and forage. Therefore, it is doubtful if the method can be used with any degree of confidence on winter range plants. However, the absorption curves

TABLE 2. AVERAGE DIGESTIBILITY AND DRY MATTER INTAKE OF WINTER RANGE FORAGES CALCULATED BY THE LIGNIN RATIO TECHNIQUE AS COMPARED TO THE CHROMOGEN METHOD

Forage species	Number of animals	Average animal weight	Lignin		Chromogen		Dry matter intake		Dry matter digestibility	
			Forage	Feces	Forage	Feces	Lignin technique	Chromogen method	Lignin technique	Chromogen method
		pounds	percent	percent	units/gm.	units/gm.	lbs./day	lbs./day	percent	percent
Big sagebrush	6	146	11.9	24.1	177	52	3.17	0.45	50.5	—240.0
Shadscale	6	153	12.4	24.1	59	104	2.94	2.66	48.8	43.3
Winterfat	2	150	8.1	11.5	74	86	3.31	2.71	29.3	13.8
Black sage	2	131	16.1	27.0	120	104	3.07	1.59	40.3	—15.3
Squirreltail grass	2	142	8.2	15.4	12	40	4.09	6.94	47.9	68.7
Alfalfa		...	7.9	21.1	147	374	....	....	61.9	60.7

of the chromogen material in alfalfa and feces from alfalfa hay at 406 mμ was in close agreement with the various curves presented by Reid *et al.* (1950). Because of this fact, the chromogen method may

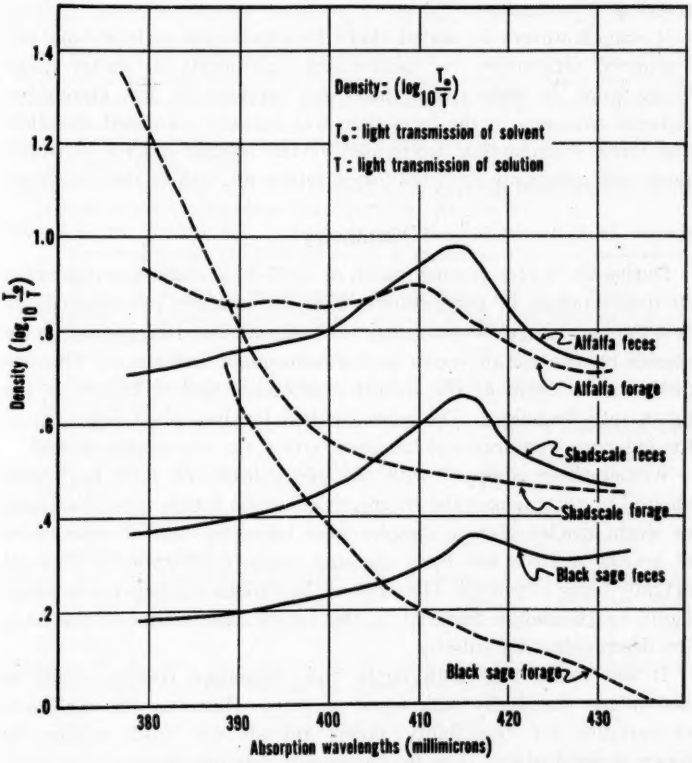

Figure 2. Absorption curves for alfalfa hay, two range forages and for the feces of sheep after they consumed these plants. Different amounts of plant and feces material were used in the chromogen determination. Therefore, the curves do not show quantitive measurements of chromogen in these materials.

have some value for determining the digestibility of some pasture species.

It is realized that it would be desirable to compare the two methods with conventional digestion trials where the intake of forage and nutrients can be measured in weight units of feed intake. However,

sheep in digestion crates do not consume winter range plants in the same manner as they normally select their forage on the range. Often times the animals reject the collected material entirely. Therefore controlled feeding trials with native forage plants have not been found practical.

It can, however, be stated that the chromogen method was not considered satisfactory for determining digestibility of winter range forage since, in some cases, there was considerably less chromogen material recovered in the feces than was actually consumed. In addition forage consumption determined by this method did not generally agree with commonly accepted forage intake for various sizes of sheep.

Summary

During the winter grazing season of 1950–51 a study was conducted on desert ranges in northwestern Utah to compare two methods of determining forage consumption and digestibility of native forage species by the use of tracer or indicator plant substances. The one method uses lignin as the indicator substance and is known as the lignin ratio technique. The other method involves plant pigments as the indicator substance and has been termed the chromogen method.

Wether sheep equipped with specially constructed fecal bags were allowed to graze temporary enclosures on native forage types composed of single species. Forage samples were taken by careful observation of grazing animals and hand plucking forage comparable to material actually being consumed. The ratio of the various constituents to either lignin or chromogen material in the forage and feces was the basis for determining digestibility.

It was found that both lignin and chromogen content could be determined chemically with equal accuracy. However, the coefficient of variation for digestibility among animals was much smaller by lignin determinations than by chromogen determinations.

Forage consumption as determined by the lignin ratio technique agreed rather closely with calculated dry matter intake commonly accepted for the various sizes of sheep. Calculated digestibility of dry matter by the use of lignin as the indicator substance compared favorably with digestibility results obtained by other investigators on similar forages. This was not generally true for determinations made by the chromogen method.

It was concluded that the chromogen method was not suited for determining digestibility of winter range forage since, in some cases,

there was considerably less chromogen material recovered in the feces than was actually consumed.

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DYE AS A REFERENCE MATERIAL FOR DETERMINING THE DIGESTIBILITY OF A RATION WITH LAMBS[1]

J. E. CORBIN[2] AND R. M. FORBES

University of Illinois[3]

MANY attempts have been made to use naturally occurring plant substances and added materials as indicators for the determination of digestibility in ruminants, yet each substance seems to possess limitations that prevent investigators from placing full confidence in it as a reference material for digestibility studies. One of the limitations in the use of naturally occurring substances such as lignin, protein, and chromogens is that they do not occur in equal concentrations in various portions of the plant, and it is logical to believe that the centers of concentration will change as the plant changes in its growth. Lignin, one of the most successfully used reference materials (Forbes and Garrigus, 1950) is concentrated in the stem while the leaves are the point of highest concentration for pigments.

Edin (1918) found that the use of chromium oxide, impregnated in blotter paper and fed to the animal in its ration, simplified considerably the methods of digestibility determination previously used. The recovery of Cr_2O_3 in 26 experiments averaged 99.1 percent with slight variation. Since the method of chromium determination used by Edin is long and complicated, two modified procedures have been developed (Schurch *et al.*, 1950) (Cullison, 1947) and applied to digestibility studies with animals.

Numerous other materials, including silicon dioxide (Gallup *et al.*, 1945) (Hodgson and Knott, 1932) iron (Knott *et al.*, 1934) (Hale *et al.*, 1939) and radioactive tracers (Hervey, 1948), have been used as indicators of digestibility. However, none of these are considered entirely satisfactory.

Preliminary investigations with dye materials gave indications that some dyes might be fed to lambs and completely recovered from the feces. The experiments reported herein were undertaken to determine the feasibility of using added dye as a reference material for determining the digestibility of a ration fed to lambs.

[1] The data are taken from a thesis submitted by J. E. Corbin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Present address: National Oats Company, East St. Louis, Illinois.

[3] Department of Animal Science, Urbana, Illinois.

Experimental

Fecal samples from lambs grazing lush green pasture were extracted with benzene and the extract subjected to a spectrophotometric determination at a series of wavelengths throughout the visible spectral range; it was found that at wavelengths near 578 mu very little light was absorbed by extracted fecal materials, thus dyes having strong absorption in this range should be used. Anthraquinone violet[4] is adapted to measurement at this wavelength and is readily soluble in benzene and easily extracted.

Method of Dye Extraction from Feces

A weighed portion (5–10 gm.) of whole or ground feces was transferred to a Waring micro-blender with benzene from a wash bottle and after enough additional benzene had been added to bring the volume to approximately 100 ml., the material was agitated for two or three minutes at a low speed. The supernatant liquid was then decanted down a stirring rod into a 400 ml. beaker and then decanted from the beaker in a similar manner into a Buchner funnel which was fitted with Whatman No. 42 filter paper that had been sealed with benzene under partial vacuum. The solution was filtered into a 1,000 ml. suction flask. The material remaining in the beaker was washed back into the blender and enough benzene was added to bring the volume to approximately 100 ml. After a second agitation for two or three minutes a transfer was effected as before. The process of agitation and filtration was repeated twice more.

The filtrate was transferred quantitatively to a volumetric flask and the volume brought to 500 ml. with benzene. This concentration was compared with two bottled control samples which had a density of 0.1 and 1.0 respectively.[5] If this concentration appeared too dense to fall within the range of 0.1 to 1.0, a 50 ml. aliquot was removed, transferred to a 100 ml. volumetric flask and diluted to volume with benzene. The optical density of this solution could be determined immediately or several hours later with concordant results. Approximately 20 minutes were required for the extraction of each sample.

Density and Dye Determination

Density determinations were made on a Beckman Model DU spectrophotometer using different known concentrations of dye dissolved in

[4] Courtesy O. Stallmann, E. I. duPont de Nemours Company, Wilmington, Delaware.
[5] The Beckman spectrophotometer is most accurate when recording a density between 0.1 and 1.0.

benzene. The dye concentration was plotted against the optical density of each solution, giving a density-concentration curve from which the concentration of dye in an unknown solution could be determined from a density measurement. The density-concentration curve for anthraquinone violet was made at 578 mu wavelength. This resulted in a straight line (figure 1) indicating that it conformed to Beer's law.

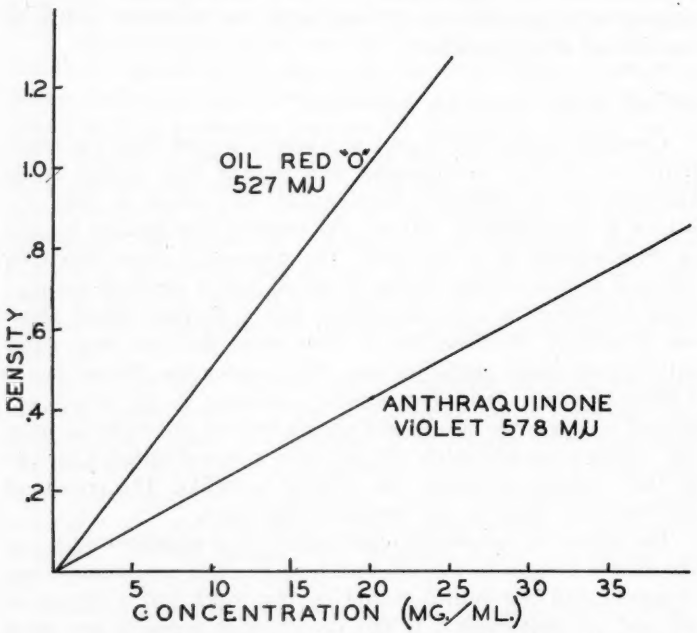

Figure 1. Density-Concentration Standard Curves Obtained in This Study.

After determining the density of an extract, the amount of dye per gram of feces was obtained by referring to the standardization curve. By multiplying the concentration of dye in the feces by the amount of feces excreted, it was possible to calculate the total excretion of dye for any given period. Use of similar extracts of feces from lambs receiving the same ration, but no dye, provided the "blank." The optical density of the blank with benzene at 0, was usually less than 0.1.

Recovery Trials

A pilot test was conducted in which a diazo dye[6] was fed to 8 lambs. The fecal recovery of dye for seven lambs averaged 81.6±2.2 percent. The eighth lamb gave a much lower recovery. This dye was discarded and a trial using anthraquinone violet as a reference material was initiated.

Eleven lambs were fed measured amounts of ground alfalfa twice daily and were given a gelatin capsule before each feed; those administered to the nine test lambs contained 0.5 gram of dye each while those for the two controls were empty. Following a preliminary period of five days, collection sacks and harness were fitted and the feces were collected for nine days, once daily for yearlings and three times daily for the lambs. The latter collections were made separately in order to study the diurnal variation of dye concentration in the feces. After drying at 65° C. until no additional loss of weight occurred, the feces were ground and triplicate samples of five grams each were removed from each total-composite sample. All of the samples were extracted with benzene as previously described and stored in sealed bottles until spectrophotometric analyses were made.

Results and Discussion

The recovery of anthraquinone violet from the feces of nine lambs is shown in table 1 and ranged from 96.4 to 106.0 percent with an average of 100.5±2.8. This represents a satisfactory degree of accuracy. Digestion coefficients calculated by the dye ratio and conventional methods would be of similar accuracy. In barn-feeding trials where the concentration of dye in the feed is known, a convenient formula for calculating digestibility is: $D=100-100\frac{x}{y}\cdot\frac{A}{B}$, where x and y=percent of nutrients in dry matter of feces and feed, respectively, and A and B=per cent of indicator in dry matter of feed and feces, respectively. Since the use of this formula does not necessitate a total collection of feces it was important to study the variation in dye concentration of feces excreted at different times in order to determine the expected error resulting from partial collections. The results of this study are shown in table 2.

Noon fecal collections composited for each lamb contained a greater dye concentration than either the morning or night collections. That

[6] Oil Red "O." Contributed by National Aniline Division, Allied Chemical and Dye Corporation.

TABLE 1. FECAL RECOVERY BASED ON TOTAL COLLECTION METHOD COMPARED WITH CALCULATED RECOVERY BY REFERENCE MATERIAL METHOD

	Yearling wethers			Wether lambs					
Lamb number	1A	2A	3A	1B	2B	3B	4B	5B	6B
Dry feces excreted, Kgm.	3.513	3.577	3.417	1.698	2.164	1.937	1.400	1.920	2.036
Dry feces excreted, based on dye recovery, Kgm.	3.495	3.791	3.461	1.658	2.138	1.883	1.424	1.974	1.996
Dye and chromogen, (mg./gm. dry feces)	2.570	2.680	2.688	5.45	4.23	4.73	6.38	4.58	4.53
Total dye and chromogens excreted, gm.[1]	9.028	9.585	9.185	8.711	9.153	9.160	8.932	8.794	9.221
Chromogen correction, gm.	.056	.057	.055	.034	.043	.039	.028	.038	.041
Dye excreted, gm.	8.972	9.528	9.130	8.677	9.110	9.121	8.904	8.756	9.180
Dye recovery, %	100	106	101	96	101	101	99	97	102

[1] Chromogen corrections of 0.016 and 0.02 mg. of dye per gram of dry feces excreted determined for the yearlings and lambs respectively by the use of control wethers not fed dye.

this variation was not large is shown by the following example: If the total daily fecal output were calculated from the noon collection of lamb 4,[7] this calculated total would be 142.8 grams or 12.7 percent less than the 164 grams actually excreted. If a sample from the morning collection were used, this calculated total would be 174.5 or 5.8 percent larger than the actual total. This variation of dye content in samples collected at morning, noon, and night indicates the desirability of adequate samples being collected at different times throughout the day.

It would appear logical to employ this dye in pasture studies with

TABLE 2. DYE CONTENT[1] (MG./GM. DRY FECES) NOON, AND NIGHT FECAL COLLECTIONS

Lamb	1	2	3	4	5	6
Morning	4.78	4.00	4.55	5.70	4.48	4.39
Noon	5.70	4.53	5.01	7.00	4.71	4.64
Night	4.81	3.83	4.27	5.89	4.12	4.53

[1] Corrected for naturally occurring chromogens absorbing light at the wavelength used.

ruminants. Digestibility of forages may be determined with a fair degree of accuracy from a study of lignin content of forages and the resultant feces. In a grazing trial, total dry matter output in the feces could be determined, without total collection, by means of studies of dye concentration in feces of animals given known amounts of the dye at frequent intervals. This would eliminate the use of collection bags and harness. The problem of identifying feces from several animals grazing in one lot may be solved readily by feeding each animal a differently colored water-soluble non-absorbed dye. This would identify the feces but would not complicate the determination of oil-soluble test dye.

During these short-time tests no evidence of a toxic action of any of the several dyes used was obtained. More detailed studies on this point and on the possible toxic action of the dyes on forage growth should be made before large scale use of the dyes is undertaken.

Summary

This experiment was designed to find a reference material that could be fed to lambs and completely recovered in the feces. With such a

[7] Lamb 4 had one of the largest variations, his morning fecal collection contained the lowest concentration of dye per gram and like the others, his noon collection contained the highest concentration.

material the use of existing formulae would permit the calculation of digestible nutrients in a ration to be made without a total collection of feces.

Anthraquinone violet dye was fed to nine lambs and the recovery of the dye from the feces ranged from 96.4 to 106.0 with an average of 100.5 ± 2.8 percent. The dye was not observed to be toxic to the animals under the conditions of the test.

A rapid and efficient method of extraction and determination of small amounts of dye in the feces of lambs is presented. This method is based on the extraction of dye with benzene and the measurement of density with a spectrophotometer. By a comparison of the density obtained with a calibrated density-concentration curve, the amount of dye extracted can be measured.

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EFFECT OF FEEDING DDT DUSTED ALFALFA HAY TO FATTENING LAMBS[1, 2]

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INVESTIGATORS in Utah and other areas (Lieberman, 1945; Smith and Michelbacker, 1946; and Lieberman and Hare, 1946) have shown that in the production of alfalfa seed, alfalfa weevil and lygus bugs are effectively controlled by the application of DDT to the alfalfa. The great increase in alfalfa seed yields, resulting from the use of DDT, has stimulated widespread interest in its possible use in alfalfa hay production. However, the question of carry-over DDT residues to farm animals through ingestion of such treated alfalfa hay has not received sufficient attention. It is important to know the amount of residue on treated alfalfa hay, and also to know the effects of DDT on livestock and upon their utilization of feed.

Orr and Mott (1945) fed between 100 and 200 mgms. of DDT per kgm. body weight to cattle, sheep and horses. Within 48 to 72 hours after the feeding of DDT began, tremors were noted in the hind quarters of some of the cattle. These symptoms continued for 3 to 6 days and then disappeared. In addition, the cattle were nervous and showed loss of appetite. Other than loss of appetite, few symptoms were noted in the other species. When the animals were slaughtered, hemorrhages were found in the heart and lining of the intestines. Fitzhugh (1948) reported that the liver of laboratory animals was damaged when diets containing only 10 ppm DDT were fed. Extensive histological changes occurred only after feeding 400 ppm DDT.

Laug *et al.* (1950) have shown that DDT accumulates in the fat of rats fed diets containing as little as 1 ppm. Maximum storage was obtained after feeding from 19 to 23 weeks. After feeding a DDT-free diet for 3 months to these rats, 25 percent of the DDT still

[1] Research supported in part by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service, and published with the approval of the director of the Utah Agricultural Experiment Station.

[2] The digestibility data were taken from a thesis submitted by Than Myint in partial fulfillment of the requirements for the M.S. degree. Part of the data were presented at the meeting of the Western Section of the American Society of Animal Production in Corvallis, Oregon, June, 1948.

[3] Position of authors, respectively: chairman, Institute of Nutrition; formerly graduate student in Animal Husbandry; professor of Physiology; professor of Chemistry; Head, Veterinary Science Department; professor of Veterinary Science; and president, Utah State Agricultural College (formerly head, Animal Husbandry Department).

remained in the fat. Hepatic cell alterations occurred from diets containing as little as 5 ppm DDT.

DDT accumulates in the fatty tissue and milk of dairy cows (Biddulph *et al.*, 1950) as well as the fatty tissue and eggs of chickens (Bryson *et al.*, 1950). The DDT dusted hay that was fed in these experiments was apparently not toxic to the dairy cows or chickens.

This investigation was conducted to determine if residual quantities of DDT on alfalfa hay caused toxic effects when such hay was fed to fattening lambs. The experiment was conducted between October 1947 and February 1948.

Experimental

Preparation of Alfalfa Hay

A 44-acre field of alfalfa was divided into 16 one-acre plots with intervening buffer strips. The buffer strips were necessary in order to reduce the drift of DDT from one plot to another during its application to the alfalfa. The plots were divided into 4 blocks of 4 plots each, and random selection was used in assigning the four treatments to the plots within each block. Technical DDT was applied to the plots at the following levels per acre: (1) none; (2) 1 pound; (3) 2 pounds; and (4) 4 pounds. These amounts of DDT provided the amount that is recommended for insect control and approximately twice the recommended amount. The DDT was mixed with pyrophyllite and applied at the rate of 20 pounds per acre with a power duster. The time of application was determined by making a count of the number of alfalfa weevil larvae *(Hypera postica Hon.)* present on the alfalfa. Dust was applied on May 29, 1947.

The hay was cut, raked into windrows, sun-cured, baled, and hauled between June 19 and July 1, 1947. This hay was designated as field-treated hay (table 1).

Feeding Trial

Experimental Design. The design is shown in table 1. The initial weight of the wether lambs varied from 57 to 80 pounds. The 16 Rambouillet and the 16 Columbia x Rambouillet lambs that weighed the most were allotted at random to block A of the field-treated and laboratory-treated hay. The remaining 16 Rambouillet and 16 Columbia x Rambouillet lambs were allotted at random to the hay from block B.

Feeding and Diets. Each block of 32 lambs was allotted at random to two groups of 32 pens. The lambs were fed in individual pens

(figure 1) but were turned together during the day for water and exercise.

The lambs were fed the alfalfa hays previously described as well as a grain mixture of the following percentage composition: dry

TABLE 1. EXPERIMENTAL DESIGN[1]

Field treated hay

Breed of lambs	DDT applied per acre	Hay replication in field 1	2	3	3
	Pounds	No. of lambs			
		Block A		Block B	
	0	1 3	1 41	1 95	1 101
Rambouillet	1	1 27	1 57	1 111	1 115
	2	1 13	1 23	1 67	1 91
	4	1 15	1 59	1 125	1 119
		Block B		Block A	
	0	1 69	1 71	1 29	1 21
Rambouillet	1	1 73	1 97	1 17	1 37
X	2	1 85	1 93	1 11	1 49
Columbia	4	1 117	1 79	1 35	1 31

Laboratory alfalfa hay

Breed of lambs	DDT fed in capsules	Blocks A		B	
	ppm of hay	No. of lambs			
	0	1 5	1 61	1 107	1 75
Rambouillet	50	1 55	1 47	1 65	1 83
	100	1 45	1 7	1 81	1 89
	200	1 25	1 19	1 103	1 87
	0	1 53	1 33	1 121	1 113
Rambouillet	50	1 63	1 51	1 99	1 77
X	100	1 9	1 39	1 127	1 105
Columbia	200	1 43	1 1	1 123	1 109

[1] One degree of freedom of field replication by breed was confounded with blocks because it was desired to divide the lambs into two groups of 32 each.

molasses beet pulp 33.0, whole barley 65.5, salt 1.0, and steamed bonemeal 0.5. Each lamb was started on a daily allowance of 0.25 pound of grain and all the alfalfa hay it would eat. Gradually the grain mixture was increased until the lambs were consuming slightly more grain than hay at the end of the feeding period. In addition,

the lambs had free access to water, iodized salt, and a mixture of one-third iodized salt and two-thirds steamed bonemeal.

The technical DDT which was fed with the laboratory alfalfa hay, was weighed into small gelatin capsules and force-fed to the lambs each day with a balling gun as outlined in table 1. The amount of DDT fed was based on the previous two weeks average alfalfa hay intake. Accurate food intakes were kept by weighing the feed daily, removing the orts daily and weighing them at two week intervals.

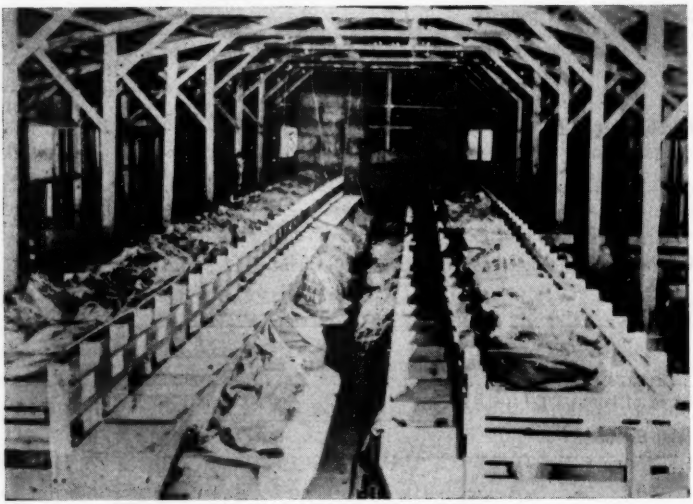

Figure 1. Individual pens and feeders with bags containing hay for each sheep, in front of the pens. Bags containing orts for each sheep are on top of the pens.

At the end of 112 days feeding, a sample of venous blood was obtained from each animal for the determination of calcium, phosphorus, hemoglobin, and DDT. The animals were then killed and samples of mesenteric and kidney fat (these were combined into a single sample which was analyzed), the lean portion of the anterior longissimus dorsi muscle, liver, kidney and brain were taken for DDT analysis.

Samples of the liver, gall bladder, kidney, cerebrum, cerebellum, medulla, hind and fore leg muscle, and thyroid were saved for histological study. All the tissues except the nervous tissues were fixed

in Zenkers solution. The latter were fixed in 10% formalin. All sections were stained with hematoxylin and eosin.

Digestion and Metabolism Trial on Field-treated Hay

A 4 x 4 Latin square design using four wethers as the columns and four periods of time as the rows, was employed to determine the digestibility of protein, ether extract, crude fiber and nitrogen-free extract of the field-treated alfalfa hay. In addition, the total digestible nutrients and metabolizable energy content were determined. The alfalfa hay diet of these sheep was not supplemented with grain.

The trials were carried out as described by Hamilton (1928) with the exception that the amount of methane was calculated by the following formula developed by Black[4] (1948):

$$\text{methane in gms.} = 2.41 \times + 9.80$$

where x represents hundreds of grams of carbohydrate digested. To convert grams of methane to Calories, the methane in grams was multiplied by 13.3444.

DDT residue on the hay and in the tissues was determined by the methods described by Harris *et al.* (1949).

Results

Yield and Chemical Composition of the Alfalfa Hay

The average DDT residue on the hay was 0, 15, 22, and 42 parts per million for the 0, 1, 2, and 4 pound levels of DDT (table 2).

There was a highly significant difference in the yield of alfalfa hay in tons per acre between the undusted and DDT-dusted alfalfa hay (table 2). However, there were no significant differences in yield among the 1, 2, and 4 pound levels of DDT. The increase in yield of alfalfa hay was no doubt the result of the protection furnished by the DDT against the alfalfa weevil and other insects.

The DDT-dusted alfalfa hay had a bright green color while that that was not dusted had a grayish-green color. The leaves on the dusted hay were whole while those on the non-dusted hay were almost completely eaten by the alfalfa weevil. Figure 2 shows these differences. A difference in quality of the hays was indicated by a higher carotene content of the DDT-dusted hays (table 2).

[4] Personal communication.

TABLE 2. YIELD, DDT RESIDUE, AND CAROTENE CONTENT OF ALFALFA HAY

DDT applied per acre	DDT residue (dry basis)	Dry matter per acre	Carotene (dry basis)
pounds	ppm	tons	ppm
0	0.0	1.12	1142
1	15	1.50	1399
2	22	1.40	1533
4	42	1.46	1561

There was a highly significant difference in percentage ether extract between the undusted and DDT-dusted alfalfa hay (table 3). The untreated hay containing 2.16 percent ether extract and the treated hays 2.46 percent, 2.46 percent, and 2.43 percent for the 1, 2, and 4 pound DDT treatments, respectively. There was no significant difference among the hays for protein, crude fiber, ash, nitrogen free extract, phosphorus, and calcium.

Feed Consumption and Live-weight Gain

There were no apparent differences among the treatments in consumption of hay and grain when the lambs were fed the field treated

Figure 2. Alfalfa from DDT dusted plots (no. 1) and from plots not dusted with DDT (no. 2). Note the damage to the plant by the alfalfa weevil larvae in the latter.

TABLE 3. PERCENT CHEMICAL COMPOSITION OF ALFALFA HAY (DRY BASIS)

DDT applied per acre	Protein	Ether extract	Crude fiber	Ash	Nitrogen-free extract	Phosphorus	Calcium
pounds	percent	percent	percent	percent	percent	percent	percent
0	15	2.2	32	9.0	42	0.22	2.1
1	16	2.5	31	9.1	41	0.23	1.9
2	16	2.5	31	8.7	42	0.22	1.8
4	16	2.4	30	9.2	42	0.23	1.9

hay or the laboratory hay. The daily gain was approximately equal for all treatments (table 4).

Blood and Tissue Analyses

It is apparent from the data in table 5 that there were no significant differences among the treatments in the amount of hemoglobin, plasma inorganic phosphorus, or plasma calcium in the blood of the lambs. No DDT could be detected in the blood of the lambs at the time of slaughter.

A detailed report of the DDT residue in the tissues of the lambs has been published (Harris *et al.*, 1949). The average amount of DDT present in the mesenteric and kidney fat was 1.9, 15.8, 18.2, and 44.2 ppm in the lambs consuming alfalfa hay dusted with 0, 1, 2, and 4 pounds of active DDT per acre, respectively. The average amount present in the mesenteric and kidney fat of lambs receiving DDT in capsules was 0, 19.6, 32.1 and 73.4 ppm when 0, 50, 100 and 200 ppm DDT, respectively, were fed. The average amount of

TABLE 4. DAILY GAIN IN WEIGHT OF LAMBS FED ALFALFA HAY TREATED WITH VARIOUS AMOUNTS OF DDT

Field hay			Laboratory hay	
DDT applied per acre	DDT residue on hay	Daily gain[1]	DDT fed in capsules	Daily gain[1]
pounds	ppm	pounds	ppm of hay	pounds
0	0	0.35	0	0.37
1	15	0.33	50	0.41
2	22	0.34	100	0.36
4	42	0.35	200	0.38

[1] Average of eight lambs.

TABLE 5. BLOOD ANALYSES OF LAMBS FED ALFALFA HAY TREATED WITH VARIOUS AMOUNTS OF DDT

DDT applied per acre	DDT residue	Hemoglobin[1]	Phosphorus[1]	Calcium[1]
pounds	ppm	gm./100 ml.	gm./100 ml.	gm./100 ml.
0	0	13	3.2	9.1
1	15	14	3.2	9.3
2	22	13	3.1	9.1
4	42	13	3.1	8.8
DDT fed in capsules				
ppm of hay				
0		12	3.3	9.1
50		12	3.4	9.2
100		13	3.3	9.2
200		14	3.3	9.1

[1] Average of eight lambs.

DDT found in the muscle, liver, kidney, and brain varied from 0 to 1.7 ppm.

Histological Analyses

There were no gross or histopathological changes in the liver, gall bladder, kidney, cerebrum, cerebellum, medulla, hind and fore leg muscle, or thyroid of the DDT-treated animals.

Digestion Coefficients and Metabolizable Energy Content of the Alfalfa Hay

The digestion coefficients were apparently not different for protein, ether extract, crude fiber, or nitrogen free extract (table 6).

The nitrogen balance was significantly in favor of decreasing amounts

TABLE 6. DIGESTION COEFFICIENTS OF ALFALFA HAY WHEN FED TO YEARLING WETHER SHEEP[1]

DDT applied per acre	DDT residue	Protein	Ether extract	Crude fiber	Nitrogen free extract
pounds	ppm	percent	percent	percent	percent
0	0	69	46	46	70
1	15	68	48	46	73
2	22	73	38	49	73
4	42	69	38	49	73

[1] Average of four wethers fed the same hay.

of DDT residue on the hay (table 7), while the metabolizable energy was significantly in favor of the DDT treated hay. There were no differences among the treatments in total digestible nutrients.

Effect of Breed

There were no differences between the breeds of sheep in their response to DDT as measured in this experiment. The other measurements taken on the breeds will be reported in another publication.

Discussion

The results reported indicate that there were no toxic effects in lambs that received alfalfa hay having a DDT residue and grain, or in lambs that received DDT in capsules with hay and grain. These

TABLE 7. NITROGEN BALANCE, METABOLIZABLE ENERGY, AND TOTAL DIGESTIBLE NUTRIENTS OF ALFALFA HAY WHEN FED TO YEARLING WETHER SHEEP

DDT applied per acre	DDT residue on day	Daily nitrogen balance	Metabolizable energy per kgm. dry matter	Total digestible nutrients
pounds	ppm	grams	Calories	percent
0	0	3.1	2108	53
1	15	1.8	2542	54
2	22	0.5	2601	56
4	42	0 1	2573	54

results agree with those of Orr and Mott (1945) in which they fed more DDT to sheep than was fed in the present study and observed no toxic effects. Laug *et al.* (1950) and Fitzhugh (1948) observed liver damage in rats with diets containing less DDT than the amount fed in the present experiment. However, they were working with a different species, and also fed their animals for a longer period of time. A marked species difference in DDT tolerance exists (Orr and Mott, 1945; Laug *et al.*, 1950).

Alfalfa hay having a DDT residue when fed without grain caused a decrease in nitrogen balance. This observation has been confirmed with growing dairy calves (Bohman, 1951). The significance of these findings cannot be stated, however, until further experiments are conducted using feeds containing DDT residue.

Summary

A 44-acre field of alfalfa hay was laid off in 16 one-acre plots with intervening buffer strips. The 16 plots were divided into 4 blocks of 4 plots each. DDT was applied to the plots at the following levels per acre: (1) none; (2) 1 pound; (3) 2 pounds; (4) 4 pounds. In addition another lot of alfalfa hay was procured to feed to lambs along with DDT in capsules at the following amounts in parts per million of hay: 0, 50, 100, and 200. Thirty-two Rambouillet and 32 Rambouillet x Columbia lambs were segregated according to breed and weight and allotted to the eight treatments at random. They were fed individually for 112 days. Digestion coefficients, total digestible nutrients, nitrogen balance and metabolizable energy were determined on 4 of the field treated plots of alfalfa hay using 4 lambs in a Latin square design. The collection period lasted 7 days.

The average DDT residue on the alfalfa hay was 0.0, 15, 22, and 42 parts per million when 0, 1, 2, and 4 pounds of DDT was applied per acre of alfalfa.

The DDT dusted alfalfa hay had a greater ether extract content, carotene content, and yield of dry matter per acre.

There were no apparent differences among the alfalfa hays in content of protein, ash, crude fiber, nitrogen free extract, phosphorus, or calcium.

There were no apparent differences among the alfalfa hays in the digestibility of protein, ether extract, crude fiber, and nitrogen free extract or in the amount of total digestible nutrients.

The DDT dusted alfalfa hays had an increased metabolizable energy content when compared to the non-dusted alfalfa hay.

There was an increase in nitrogen balance as the amount of DDT residue decreased on the alfalfa hay.

There were no apparent differences among the DDT-treated field hays or the laboratory hays in rate of gain, total gain, and feed consumption of the lambs.

The average amount of DDT present in the mesenteric and kidney fat was 1.9, 15.8, 18.2 and 44.2 ppm in the lambs consuming alfalfa hay dusted with 0, 1, 2, and 4 pounds of active DDT per acre, respectively. The average amount present in the mesenteric and kidney fat of lambs receiving DDT in capsules was 0, 19.6, 32.1, and 73.4 ppm when 0, 50, 100 and 200 ppm DDT, respectively, were fed.

The average amount of DDT in muscle, liver, kidney, and brain varied from 0 to 1.7 ppm.

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A METABOLISM CRATE FOR USE WITH SHEEP[1]

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SINCE the quantitative collection of feces and urine is of primary importance in any metabolism study, steps were taken at this laboratory to design a metabolism crate for use with sheep, of either sex, which would satisfy this requirement as well as allow the experimental subject maximum freedom of movement. Metabolism crates for sheep have been described by Hodgson *et al.* (1935) and by Briggs and Heller (1940) as modifications of the crate designed by Forbes (1915) for metabolism studies with swine. Burkitt (1940) and Sotola (1927) have used similar crates which have a complete false floor of heavy wire screen upon which the animal stands. A removable screen of smaller mesh below the false floor catches the feces and permits the urine to run through to a sloped or funnel-shaped pan which directs the flow of urine into a collection bottle beneath the crate.

Contamination of feces with urine is the main disadvantage encountered with a crate of the above type. Also, feces which fail to pass through the false floor are trampled and the small particles then pass through both screens to the pan where they contaminate the urine and obstruct its flow into the collection bottle. The urine is exposed to conditions favoring increased decomposition because some splattering and spreading of urine occurs in passing through the fine mesh of the second screen. The size of the pan and its degree of slope allow the urine to spread over a considerable area which also tends to increase the amount of decomposition taking place. To discourage the scattering of feed inside the crate, feed boxes are usually fastened on the outside of the crate and the feed made available by an opening for the animal's head at the front of the crate above the feed box. For this opening, a stanchion is sometimes used and the animal is stanchioned at feeding time. This type of feed box, when the animal is stanchioned, deprives the animal of free access to drinking water without which it is difficult to obtain maximum feed consumption.

Recently, Briggs and Gallup (1949) described metabolism stalls which they have designed for use with wethers and steers. In the case of the stall for wethers, the collection of urine is such that the move-

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ment of the animal within the stall is greatly restricted; that is, the animal must be in a position which will allow the urine to fall directly into the funnel which directs the urine into the collection bottle. Any freedom allowed the animal may result in urine falling on the wooden floor upon which the animal stands. The method for the collection of feces appears to be very satisfactory inasmuch as there is little chance for the contamination of feces with urine.

A metabolism crate which has been found to be very satisfactory for work with sheep by the Department of Animal Nutrition at the Pennsylvania State College is of the rectangular-type as used by Watson *et al.* (1943) and by Crampton and Whiting (1943). It has been modified so that all feces pellets and urine are confined completely within the crate while they are being separated and conveyed into individual receptacles by accessory equipment which has been designed for that purpose. Spattering of urine has been reduced to a minimum by having it fall on a sloping surface rather than on a flat surface. These modifications have also made it possible to omit the harnessing of the experimental subject and to restrict the movements only to the over-all, inside dimensions of the crate.

Description of Crate

The crate proper, essentially the same as that used by Watson *et al.* and Crampton and Whiting, is shown in figures 1, 2, and 3. The height of the crate is increased to provide greater clearance between the floor of the crate and its base and to give more height to the crate itself. The opening at the rear of the crate is fitted with a door the inside of which is lined with sheet metal. The hinges on the feed box are replaced with open-slotted pivots to permit easy removal of the feed box when it is desired to remove the animal from the crate. This latter arrangement facilitates greatly the removal of the animal from the crate because of the great dislike that sheep have for backing-out of it. Three sets of holes for the rod supporting the feedbox provide for an easy adjustment of the length of the crate. To prevent the animal from turning around in the crate, the width can be varied by means of an adjustable baffle located at the side opposite the watering trough. In order to provide increased freedom of movement, the water trough is on the outside rather than on the inside of the crate. This also eliminates the possibility of having water spilled into the collection of urine since the trough can not be bumped by the animal. The opening in the side of the crate, closed by a spring-hinged door, gives the animal access to

water at all times. The spring-hinged door also serves an important function in that the additional effort required of the animal to obtain a drink of water discourages the practice of moving his head unnecessarily from the feed box to the water trough which may result in undue contamination of the water with feed.

The top of the crate is covered with 1″ x 2″ mesh hardware cloth while the sides are enclosed with one-fourth-inch mesh hardware cloth to prevent feces pellets from bouncing out of the crate. Toward the

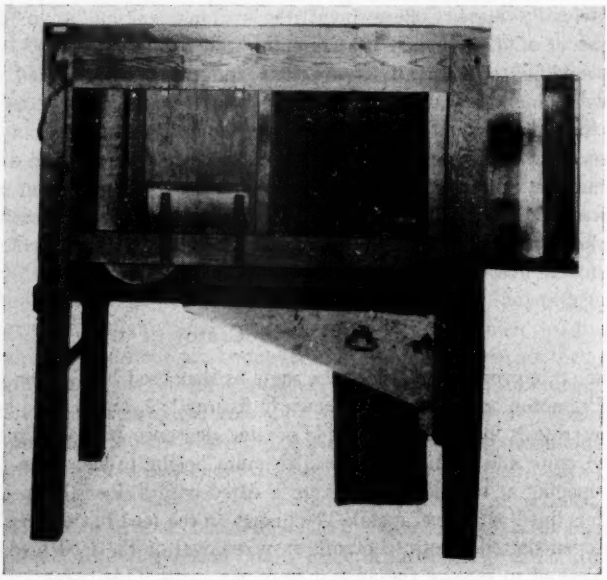

Figure 1. Side view of metabolism crate showing how the location of the feedbox determines the length of the crate and the collection tray in operating position.

bottom and on each side of the crate, sloping baffle boards, wood covered with sheet metal, are installed, the bottom edges of which are almost in contact with the floor of the crate. The bottom edge of the sheet metal covering the door is made to provide a similar effect at the rear of the crate. These devices are most satisfactory in preventing any loss of feces pellets.

In order to bring the accessory equipment, for the collection and separation of feces and urine, as close as possible to the false floor of

1″ x 1″ mesh, extra heavy, hardware cloth[2] upon which the animal stands, the horizontal wooden pieces which formerly supported the false floor, are replaced with angle iron. As is shown in figure 4, the tray for separation and collection of urine and feces is made of sheet metal and is designed to provide the maximum slope to the two false

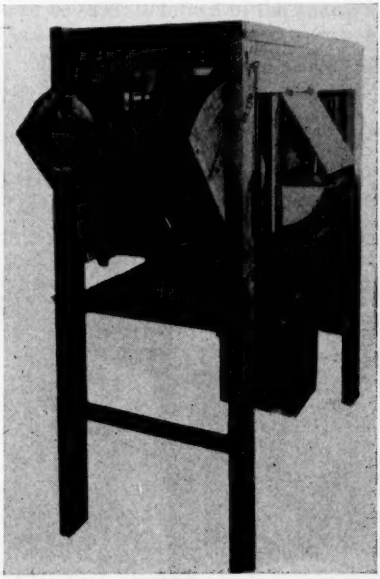

Figure 2. Front view of metabolism crate illustrating the all-metal construction of the inside of the feed box and the accessibility of the watering trough.

bottoms and the bottom of the tray. The false bottom or screen near the rear of the tray, upon which most of the feces pellets fall, has considerable slope while the second screen which lies below the first has appreciably less slope. The latter extends almost to the front end of the tray and its purpose is to catch any feces pellets which might miss the steeply inclined screen. Both screens direct the pellets into the receptacle by means of a tube which is two inches in diameter. To prevent the urine from creeping across the meshes of the upper screen

[2] Plain steel wire cloth, 1″ x 1″ mesh, 0.135″ diameter. John A. Roebling's Sons Company, 12 South Twelfth Street, Philadelphia 7, Pennsylvania.

and into the feces, an angle-iron (made of sheet metal), one-half inch by 4 inches, is soldered to the bottom side of the screen at the lowest point. The urine passes through the screens and flows immediately to lowest point at the bottom of the tray to which is connected a tube two inches in diameter that conveys the urine into the receptacle. The receptacles for the feces and urine set on a small platform suspended

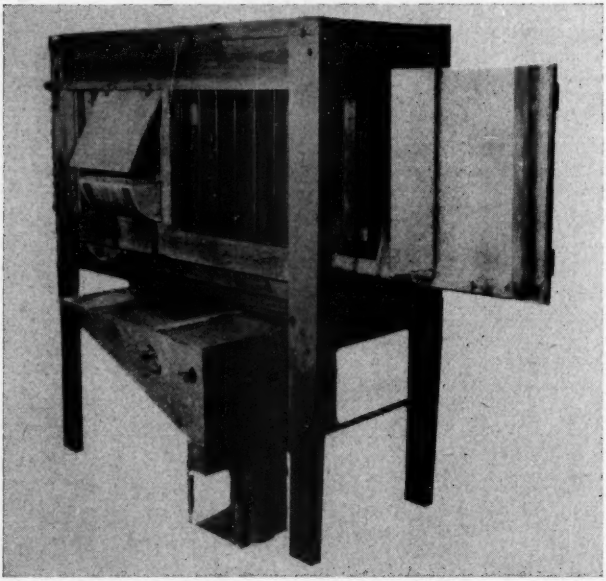

Figure 3. Rear view of metabolism crate showing the metal baffles on the door and the sides and the collection tray in position for washing the screens with hot water.

from the bottom of the tray so that the tray can be pulled out at least half-way in order to wash down the urine with hot water as frequently as desired.

A slight modification of the above collection tray becomes necessary when it is desired to make collections of feces and urine from ewes. A sloping sheet metal baffle is inserted at the back of the tray which diverts the urine on to the highly sloped screen so that it can pass through to the bottom of the tray. This baffle is shown in figure 4. There is sufficient clearance between the lower edge of the baffle and

the screen to permit the passage of the feces pellets into the proper receptacle.

To prevent the loss of any feed that the animal might spill, a sheet metal tray is made which slides in directly underneath the false floor and closes in the area between the front of the collection tray and the feed box as well as covering the front end of the collection tray. Any spilled feed can then be easily returned to the animal's feed box.

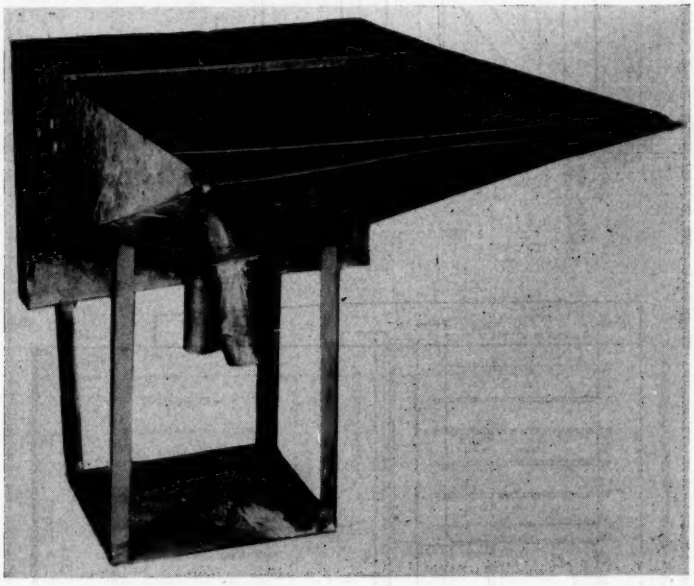

Figure 4. Collection tray illustrating the screen baffles for the separation of feces from urine and the metal baffle at the rear for use of the tray with ewes.

All dimensions of the crate and collection tray are shown in drawings made to scale and are exhibited as figures 5, 6, and 7.

The metabolism crate which has just been described has been in use at this laboratory for the past three years. During this period of time, more than one hundred and fifty 11-day collection periods have been completed which were highly satisfactory with regard to the separation of feces from urine and the quantitative collection of each. Only wethers were used during the course of these experiments.

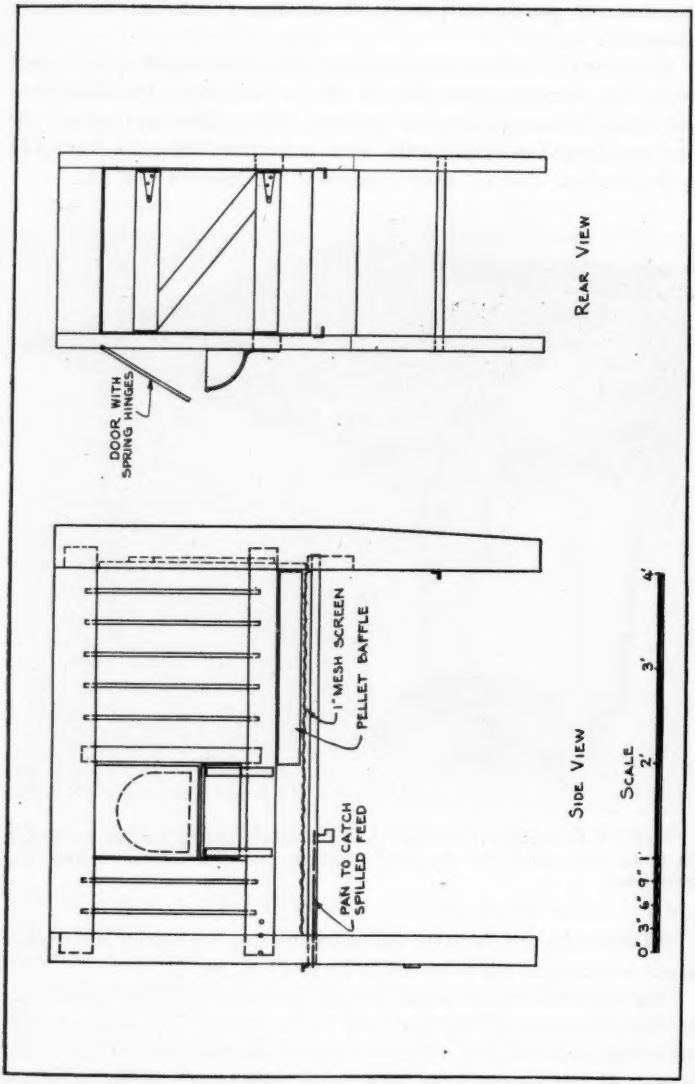
REAR VIEW
DOOR WITH SPRING HINGES
SIDE VIEW
1" MESH SCREEN
PELLET BAFFLE
PAN TO CATCH SPILLED FEED
SCALE
0' 3" 6" 9" 1' 2' 3' 4'

Figure 5

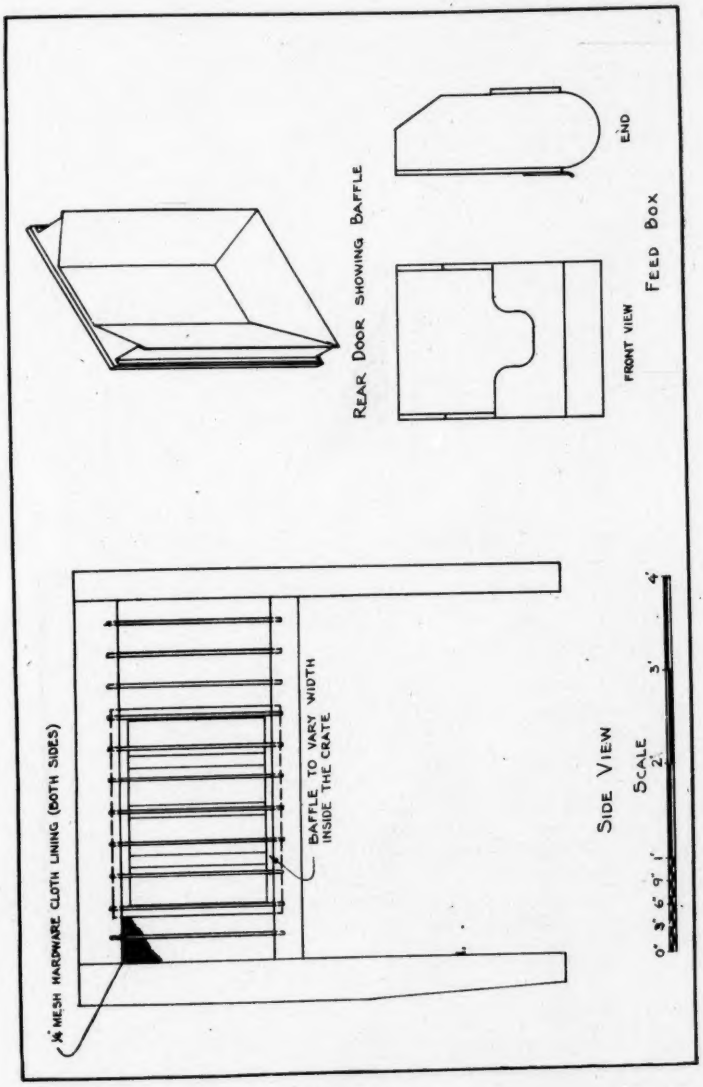
REAR DOOR SHOWING BAFFLE
END
FRONT VIEW
FEED BOX
BAFFLE TO VARY WIDTH INSIDE THE CRATE
¼" MESH HARDWARE CLOTH LINING (BOTH SIDES)
SIDE VIEW
SCALE
0' 3' 6' 9' 1' 2' 3' 4'

Figure 6

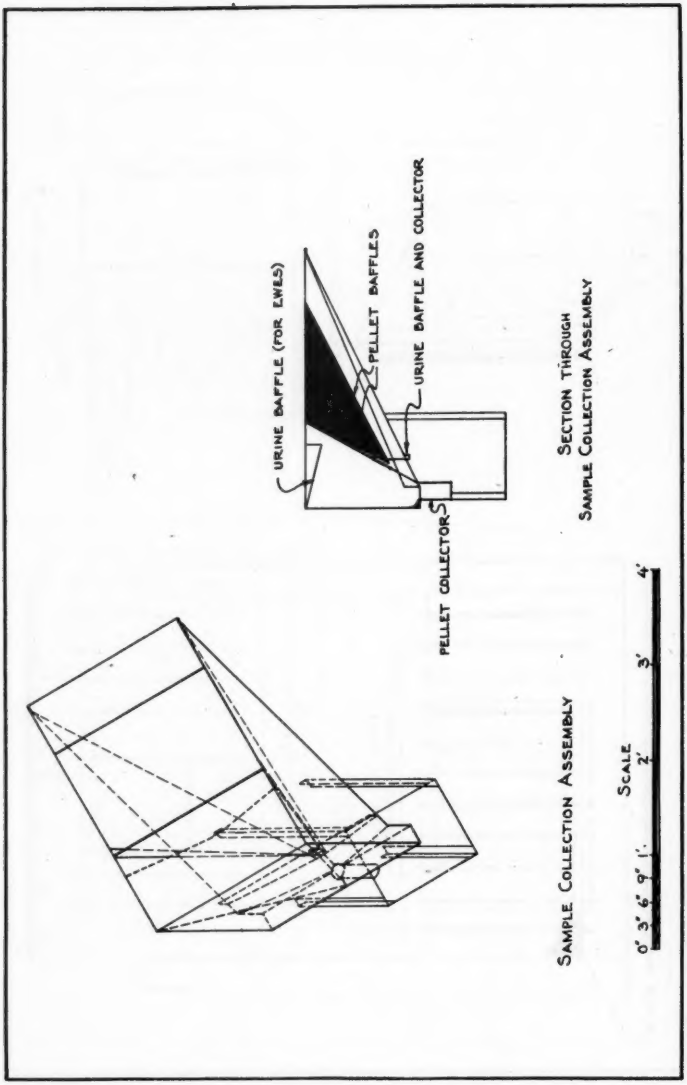
URINE BAFFLE (FOR EWES)
PELLET BAFFLES
URINE BAFFLE AND COLLECTOR
PELLET COLLECTOR
SECTION THROUGH
SAMPLE COLLECTION ASSEMBLY
SAMPLE COLLECTION ASSEMBLY
SCALE
0' 3' 6' 9' 1'. 2' 3' 4'

Figure 7

Summary

The construction of a metabolism crate is described which provides for the quantitative separation and collection of feces and urine eliminated by sheep of either sex. The metabolism crate, including the tray for the collection and separation of feces and urine, is illustrated by photographs and labelled drawings made to scale.

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THE EFFECTS OF STILBESTROL ON SUCKLING LAMBS[1]

T. W. Perry, F. N. Andrews, and W. M. Beeson[2]

Purdue University Agricultural Experiment Station

SUBCUTANEOUS implantation of stilbestrol pellets in growing and fattening animals in the feed lot has resulted in significantly increased growth rate and increased feed efficiency with lambs, (Andrews *et al.*, 1949a, 1949b; Jordon, 1950), with both beef heifers and steers, (Dinusson *et al.*, 1950, Andrews, *et. al.*, 1950) and with broilers (Andrews and Bohren, 1947, Detwiler *et al.*, 1950). However, Jordan and Dinusson, 1950, reported no growth stimulation when suckling lambs on pasture were injected with stilbestrol. This experiment was designed to investigate the effect of subcutaneous implantation of stilbestrol pellets into suckling lambs running with their dams on pasture.

Materials and Methods

Native wether and female suckling lambs (Hampshire rams x range ewes) averaging 46 pounds in weight and ranging from 21 to 42 days of age were divided equally into three groups according to sex, age, type, weight and general thriftiness. A total of 97 wether and 100 female lambs were included in this trial in which the lambs and their dams grazed together on mixed legume grass pasture. No grain was fed to the ewes or lambs. The animals remained out of doors at all times and had free access to water and a simple mineral mixture composed of 2 parts of bonemeal and 1 part iodized salt. The trial was started May 2, 1950, and was terminated August 22, 1950, a period of 112 days.

The following experimental treatments were employed: Group I, 33 wethers and 32 ewe lambs which received no injection and served as the Control; Group II, 32 wethers and 34 ewe lambs all of which were injected subcutaneously with a 12 mg. pellet of stilbestrol; Group III, 32 wethers and 34 ewe lambs all of which were injected with a total of 24 mg. (two 12 mg pellets injected simultaneously) of stilbestrol. The stilbestrol was implanted subcutaneously in pellet form in the neck region at the beginning of the experiment.

[1] Contribution from the Department of Animal Husbandry, Journal Paper, No. 516. Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] The authors express their appreciation to Wick and Fry, Inc., Cumberland, Indiana, for furnishing the stilbestrol.

The lambs were weighed individually at the beginning of the experiment and at 28-day intervals during the trial. All lambs that had reached a market weight of 90 pounds at the end of any 28-day interval were taken off the experiment and marketed. All animals failing to reach 90 pounds in weight by the end of the fourth 28-day interval were separated from their mothers and were fed shelled corn on bluegrass pasture.

TABLE 1. THE EFFECT OF STILBESTROL ON THE GAINS OF SUCKLING LAMBS ON PASTURE

Experiment I—1950 (112 days)

	Lot I Control			Lot II 12 mg. Stilbestrol			Lot III 24 mg. Stilbestrol		
	♂[1]	♀	Ave.	♂[2]	♀	Ave.	♂[1]	♀	Ave.
No. of lambs	33	32	65	32	34	66	32	34	66
Av. Initial wt.	46	46	46	46	45	46	47	45	46
Total gain/lamb	40	40	40	42	45	43	43	44	43
Av. daily gain	.44	.45	.44	.49	.54	.52**	.54	.54	.54**
No. days to reach final wt.[1]	94	93	93	89	86	87	84	83	83
No. marketed 2nd 28 days	5	7	12	7	9	16	10	8	18
No. marketed 3rd 28 days	7	8	15	12	14	26	10	15	25
No. marketed 4th 28 days	3	1	4	2	4	6	3	4	7
No. under 90 lb. after 112 days	16[3]	16	33	11	7	18	8[4]	5[5]	13

** Significantly greater average daily gain than the controls. The least significant difference for average daily gain at the 1% level is 0.05 lb.

[1] After the 112-day grazing season, 32 lambs in lot 1; 18 lambs in lot 2; and 13 lambs in lot 3 had failed to reach a market weight of 90 pounds and were taken off experiment and placed in the feedlot.

[2] All males in each lot were castrates.

[3] Two wether lambs died.

[4] One wether lamb died.

[5] Two female lambs died.

Results and Discussion

Growth Rate

The effect of implanted stilbestrol, at two levels, on the growth of suckling lambs on pasture is summarized in table 1. Excellent gains (0.44 lb. per day) were made by the animals in the control group, but the rate of gain (0.52 lb. and 0.54 lb.) was significantly greater for the lambs grazing on the same pasture and which had been subcutaneously implanted with either 12 or 24 mg. of stilbestrol. There was no significant difference in response between the two levels of stilbestrol. The greatest difference in average daily gain between the control and the treated groups occurred during the first 28-day period (control, 0.54

lb.; 12 mg. stilbestrol, 0.64 lb.; 24 mg. stilbestrol, 0.69 lb.) whereas during subsequent weigh periods the gains in all lots were lower and the difference between the control group and the treated groups was much less (figure 1). The average daily gains for the three respective

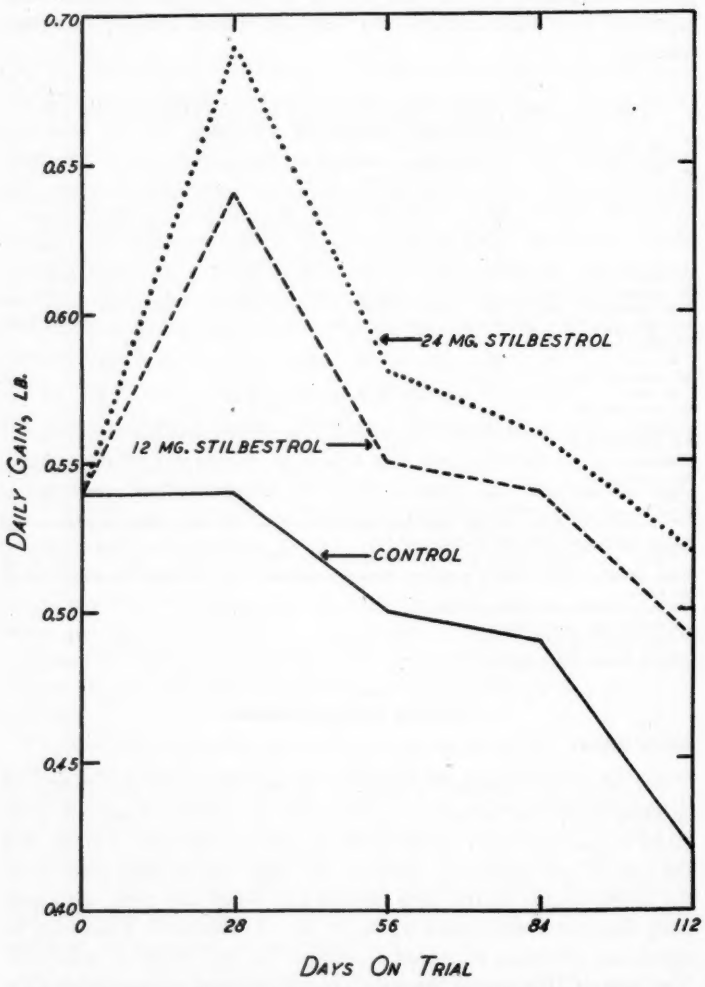

Figure 1. Effect of stilbestrol on the cumulative average daily gain of lambs running with their dams on pasture.

groups are: second 28-day period, 0.46 lb.; 0.47 lb., and 0.46 lb.; third 28-day period, 0.47 lb., 0.52 lb., and 0.51 lb.; and fourth 28-day period, 0.09 lb. 0.12 lb., and 0.14 lb. This decrease in difference between groups may be attributed to the fact that milk production in the ewes probably had begun to decline by the end of the second 28-day period. These results compare favorably with those obtained by Andrews *et al.* (1949a, 1949b) who reported stimulated growth in feeder lambs which had been implanted with either 12 or 24 mg. of stilbestrol.

At the end of 112 days on trial 15 wether lambs and 16 ewe lambs, or a total of 48 percent of the lambs, in Group I had reached a market weight of 90 pounds, in Group II 21 wether and 27 ewe lambs, or 73 percent, had reached the market weight, and in Group III 23 wether and 27 ewe lambs, or 76 percent, had reached market weight, indicating the difference in rate of gain between the control group and the treated groups.

Jordan and Dinusson (1950) were not able to stimulate the growth rate of suckling lambs on pasture by the implantation of 12 mg. of stilbestrol in twin lambs in which the response of both sexes were studied. However, the lambs used in the South Dakota trial weighed approximately 25 pounds at the time of injection whereas the data reported in this paper was from lambs that weighed approximately 46 pounds at the time of injection, indicating age and weight at time of injection may affect the results.

Sexual Development

Within two weeks after the implantation of stilbestrol under the skin of the treated lambs there was a marked development of the external genitilia of the female lambs. The vulvae became enlarged and vascular and the lambs appeared to be in estrus. While attaining the first weights 28 days following stilbestrol implantation, it was observed that many of the treated wether and female lambs evidenced mammary stimulation and that in some several streams of apparently normal milk could be expressed from each nipple. No plans for a systematic study of mammary activity had been made, but a crude estimate that at least one-third of the hormone treated lambs showed mammary secretion would be reasonably conservative.

All lambs were carefully examined at the second weigh period 28 days later and no evidence of secretion were seen at that time. Both sexes appeared to be similarly affected.

It has been clearly established that estrogen administration will induce mammary growth in many species and that in some species

copious lactation occurs following estrogen treatment. Reviews of such research have been made by Turner (1939), Lewis and Turner (1942), Trentin and Turner (1948) and others.

Summary

The subcutaneous implantation of either 12 or 24 mg. of stilbestrol significantly increased ($<0.01\%$) the gains of suckling ewe and wether lambs which were running with their dams on pasture. There was no significant difference in response between the two levels of stilbestrol.

Treated lambs of both sexes exhibited marked mammary development during the first 28 days of the 112-day trial.

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PROTEIN EFFICIENCY OF CORN BY-PRODUCTS AND OTHER PROTEIN FEEDS FOR SWINE GROWTH

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Purdue University Agricultural Experiment Station[2]

THE nutritive value of proteins for swine is of fundamental importance both for rapidity of growth and for economical production.

Corn is generally regarded as an inadequate source of protein for young growing animals because it is limited by its low tryptophan and lysine content (Csonka, 1939; Marais and Smuts, 1940; Jones *et al.*, 1948.) Close to one-fifth of the nation's corn crop is processed industrially each year and about one-fourth of the processed corn is returned to the farms in the form of by-product feeds which contain medium to high amounts of protein.

Corn oil meal (wet milled corn embryo less part of the oil) is one of the corn by-product feeds that has been shown to contain high quality protein for small laboratory animals (Osborne and Mendel, 1918; Hove *et al.*, 1945); and yet in feeding trials with swine, the corn oil meal has failed to show much promise as a swine protein supplement (Skinner and Starr, 1918; Robison, 1939).

Corn solubles (dried corn steep water solubles) contains the soluble nutrients that were removed from the grain during the soaking process of the wet milling of corn (Morrison, 1949). Although it is readily digestible, it has a very low biological value for rats (Schulz and Thomas, 1949).

Recently, various APF (animal protein factor) concentrates supplying only negligible amounts of protein have been used successfully as sources of unknown growth factors for swine (Beeson and Bloss, 1949; Hogan and Anderson, 1949; Johnson and Neuman, 1949). By using these concentrates in the rations plus all other known nutrients required by the pig, protein feeds may be more accurately evaluated for protein quality alone in contrast to measuring total nutrient effect when nutrients other than protein are not balanced or optimum in all rations.

[1] The authors wish to express their appreciation to the Corn Industries Research Foundation for the very generous grants of both money and supplies necessary to carry on this project. Dr. Geurin is now emp oyed in the Ralston Purina Livestock Research Department, St. Louis, Missouri. Dr. Hoefer is now in charge of Animal Nutrition Research at Michigan State College, E. Lansing, Michigan.

[2] Contribution from the Department of Animal Husbandry, Journal Paper No. 505, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

The following studies present the results of protein efficiency tests with young growing pigs when fed diets containing protein from either whole egg, skimmilk, corn oil meal, corn solubles, corn gluten meal, tankage, or soybean oil meal.

First Trial

Experimental Procedure. The quality of protein in the various experimental feeds was tested by a modification of the rat growth method

TABLE 1. COMPONENTS OF EXPERIMENTAL RATIONS (FIRST TRIAL)

Ingredients	Lot number and treatments		
	1 Whole egg	2 Corn oil meal	3 Corn solubles[1]
	percent	percent	percent
Protein source[2]	22.1	45.5	33.4
Dextrose	67.5	41.1	48.7
Corn oil	—	7.3	7.4
Corn fiber	4.3	—	4.4
Whole liver powder	1.0	1.0	1.0
Mineral mixture[3]	5.0	5.0	5.0
Vit. A and D oil	0.1	0.1	0.1
Vitamin mixture[4]	—	—	—

[1] Corn solubles in the dry state is very hygroscopic and becomes sticky when moisture is absorbed.

[2] The dried whole egg (Crude Protein 47.5%) was obtained from Armour & Company, Chicago, Illinois; the corn oil meal (Crude Protein 23.1%) was a solvent extracted meal from Clinton Industries, Inc., Clinton, Iowa; and the corn steep water solubles (Crude Protein 31.5%) was a dried product from American Maize-Products Co., Robie, Indiana.

[3] Heinemann *et al.* (1946).

[4] A water-alcohol solution of vitamins was mixed in the evening feed of each pig. See Beeson *et al.* (1949).

originally proposed by Osborne *et al.* (1919). In this method the protein efficiency is expressed as pounds gained per pound of protein eaten. Dried whole egg served as the source of protein in the control rations.

Fifteen weanling Duroc pigs were sorted into blocks of three pigs each by considering litter, sex and weight. Pigs in each block were then randomly placed on one of the three rations and started on experiment on November 28, 1948.

The rations fed to the three lots of pigs are presented in table 1. Partial analysis of the individual protein feeds was used to equalize the protein, fat and fiber levels of each ration at 11, 7.7 and 4.6%, respectively.

The pigs were placed on raised wire screens and housed individually in indoor pens (4 x 7 feet). Feed and water were available to the pigs at all times. Weekly feed intakes and live weights were recorded. The feed and growth data were treated statistically by analysis of variance as outlined by Snedecor (1946).

When it became obvious after 21 days of treatment that the 5 pigs receiving the corn solubles as a sole source of protein would not long survive under that dietary regime, it was decided to alter the ration by (1) doubling the level of protein, (2) supplementing with pure amino acids, or (3) adding various natural protein feeds to replace

TABLE 2. RESULTS OF FEEDING SWINE RATIONS CONTAINING EITHER DRIED WHOLE EGG, CORN OIL MEAL OR CORN SOLUBLES

Items	Lot numbers and treatments		
	1 Whole egg	2 Corn oil meal	3 Corn solubles
No. of pigs	5	4	5
No. of days[1]	35	35	21
Av. initial wt., lb.	36.0	35.5	36.0
Av. final wt., lb.	77.7	69.8	34.9
Av. gain, lb.[2]	41.7	34.3	—1.1
Av. daily gain, lb.	1.19	0.98	—0.05
Av. total feed, lb.[3]	161.1	145.1	66.2
Av. daily feed, lb.	4.60	4.15	3.15
Feed per cwt. gain, lb.	386.0	424.0	—
Protein efficiency[4]	2.35	2.14	—0.15

[1] Lot 3 pigs were removed from the experiment on December 19, 1948. Least significant difference for P=5% and 1% respectively for the following footnotes was:
[2] 5.9 and 8.7 lb.
[3] 5.4 and 8.0 lb.
[4] 0.28 and 0.42 lb.

one-half of the protein of corn solubles. The pigs that had been receiving the corn solubles ration were then transferred individually to one of the above treatments.

Results. The results obtained in the first feeding trial are summarized briefly in table 2. Dried whole egg protein supported faster growth of young weanling pigs than did either corn oil meal or corn solubles protein. The pigs receiving corn solubles lost weight and had to be removed from the experiment after 21 days. Since the source of protein was essentially the only known variable in these rations, and since the pigs receiving corn solubles were eating a fair amount of feed for their

weight, the failure to grow or even maintain body weight is presumptive evidence of poor protein quality. Further evidence was obtained when growth was progressively stimulated by adding certain amino acids. Tryptophan supplementation stimulated some growth while tryptophan plus methionine gave an additional growth response. Soybean oil meal proved effective in replacing one-half of the corn solubles protein.

Lot 1 pigs receiving whole egg as the sole source of protein grew about 21% faster and showed a protein efficiency value 10% higher than the pigs receiving corn oil meal. Observations recorded throughout the experiment indicate that the corn oil meal ration was not as palatable as the whole egg ration.

The trial was cut short when all 5 of the pigs receiving the whole egg ration developed a severe deficiency syndrome characterized by (1) greatly reduced appetite, (2) cessation of growth, and (3) external appearances similar to conditions of biotin deficiency as reported by Cunha *et al.* (1946), Lindley and Cunha (1946), Cunha *et al.* (1948). In a subsequent test using 3 pigs, the deficiency syndrome was again produced in 2 pigs receiving the egg ration and prevented in one pig that received the egg ration plus a daily supplement of 100 micrograms of biotin.

Second Trial

Experimental Procedure. The second feeding trial was conducted during the summer and was similar to the first trial in design. Dried skimmilk served as the source of protein for the controls and comparisons were made with corn oil meal, soybean oil meal, and a combination of soybean oil meal and corn solubles.

The experimental rations are presented in table 3. Each ration was balanced to contain 11% crude protein, 5% crude fat, and 4.9% crude fiber.

Sixteen weanling pigs (4 pigs from each litter) were used in this trial with litter mates being randomly assigned to each of the four test rations.

Results. Dried skimmilk protein supported faster growth of young weanling pigs than did either corn oil meal, soybean oil meal or corn solubles-soybean oil meal (see table 4). The difference of 20 pounds more average gain for the skimmilk pigs over the next best lot was highly significant (1% level).

There was no difference between the growth rates of pigs receiving corn oil meal or soybean oil meal. Both of these groups of pigs not only grew at a very similar rate throughout the 10 weeks of the experiment,

TABLE 3. COMPONENTS OF EXPERIMENTAL RATIONS[1] (SECOND TRIAL)

	Lot numbers and treatments			
	1 Skim-milk	2 Corn oil meal	3 Soybean meal	4 Corn solubles and soybean oil meal[2]
	percent	percent	percent	percent
Dried skimmilk	30.1	....	....	....
Corn oil meal	....	48.2	....	....
Soybean oil meal	....	....	24.3	12.2
Corn steep water solubles	....	....	....	18.4
Dextrose	55.5	42.0	63.8	56.2
Corn oil	4.3	4.6	3.2	3.9
Corn fiber	4.9	....	3.5	4.1
Mineral mixture	5.0	5.0	5.0	5.0
Vitamin A and D oil	0.1	0.1	0.1	0.1
Vitamin B_{12} concentrate[3]	0.1	0.1	0.1	0.1
Vitamin mixture[4]	....	....	....	....

[1] The dried skimmilk (Crude Protein 36.5%) was a roller process, non-fat, dried solids obtained from Sunshine Farms, Lafayette, Ind.; the corn oil meal (Crude Protein 22.8%) was a solvent meal from Clinton Industries, Inc., Clinton, Iowa; the soybean oil meal (Crude Protein 45.2%) was an expeller process meal from Ralston Purina Co., St. Louis, Mo.; and the corn steep water solubles (Crude Protein 29.9%) was a dried product from American Maize-Products Co., Robie, Ind.

[2] Corn solubles and soybean oil meal supplied equal parts protein.

[3] Merck No. 3 containing 12.5 milligrams of B_{12} activity per pound.

[4] One-third more than proposed recommendations (in press) of the Committee on Animal Nutrition of the National Research Council. Personal communication.

TABLE 4. RESULTS OF FEEDING SWINE RATIONS CONTAINING EITHER DRIED SKIMMILK, CORN OIL MEAL, SOYBEAN OIL MEAL OR CORN SOLUBLES AND SOYBEAN OIL MEAL

	Lot numbers and treatments			
Items	1 Skim-milk	2 Corn oil meal	3 Soybean oil meal	4 Corn solubles and soybean oil meal[1]
No. of pigs	4	4	4	4
No. of days	70	70	70	70
Av. initial wt., lb.	38.8	39.0	38.3	38.8
Av. final wt., lb.	139.3	118.4	119.4	84.8
Av. gain, lb.[2]	100.5	79.4	81.1	46.0
Av. daily gain, lb.	1.44	1.13	1.16	0.66
Av. total feed, lb.[3]	378.1	275.6	338.4	244.9
Av. daily feed, lb.	5.40	3.94	4.83	3.50
Feed per cwt. gain, lb.	376	347	417	532
Protein efficiency[4]	2.42	2.62	2.18	1.71

[1] Corn solubles and soybean oil meal supplied equal parts protein.

Least significant difference for P=5% and 1% respectively for the following footnotes was:

[2] 10.6 and 15.2 lb.

[3] 68.9 and 99.0 lb.

[4] 0.27 and 0.38 lb.

but also both made very satisfactory growth on a single source of protein.

The skimmilk ration was very palatable and the pigs on skimmilk consumed 37% more feed than comparable pigs receiving corn oil meal and 12% more than pigs receiving soybean oil meal.

When growth rates are compared per unit of protein consumed, corn oil meal protein was equal to skimmilk protein and significantly better than soybean oil meal. The average protein efficiency value for corn oil meal was slightly above that of skimmilk.

The pigs receiving corn solubles-soybean oil meal (lot 4) grew at a sub-optimum rate and showed a protein efficiency value that was

TABLE 5. COMPONENTS OF EXPERIMENTAL RATIONS[1] (THIRD TRIAL)

Ingredients	Lot numbers and treatments			
	1 Skim-milk	2 Corn oil meal	3 Tankage	4 Corn gluten meal
	percent	percent	percent	percent
Protein source	30.1	48.2	19.4	27.0
Dextrose	55.4	41.9	68.4	60.2
Corn oil	4.3	4.6	2.6	4.2
Corn fiber	4.9	....	4.3	3.3
Mineral mixture	5.0	5.0	5.0	5.0
Vitamin A and D oil	0.1	0.1	0.1	0.1
Vitamin B_{12} concentrate[2]	0.2	0.2	0.2	0.2
Vitamin mixture[3]	....	....	....	....

[1] The dried skimmilk and corn oil meal were from the same lot of feed used in the second feeding trial. See table 3; the tankage (crude protein 56.7%) was a commercial product from Kingan and Co., Indianapolis, Ind.; and the corn gluten meal (crude protein 40.9%) was a commercial product from American Maize-Products Co., Robie, Ind.

[2, 3] See footnotes [3] and [4] on table 3.

significantly lower than for any of the other groups of pigs. However, the protein efficiency value, which was 78% as high as for straight soybean oil meal, indicated a supplementary relationship between the two feeds since corn solubles alone returned a minus value in the first trial.

Third Trial

Experimental Procedure. The experimental design was essentially the same as presented in the second trial. Tankage was fed in order to bring in a comparison of one of the widely used animal protein supplements. Corn gluten meal was used instead of the corn solubles ration. The experimental rations presented in table 5 were each balanced to contain 11% crude protein, 5% crude fat, and 4.9% crude fiber.

Sixteen Duroc weanling barrows were stratified on the basis of weight and randomly assigned to one of the four test rations. The experiment was started October 28, 1949.

Results. Excellent daily gains were made by pigs receiving either skimmilk or corn oil meal as the sole source of protein in the ration (see table 6). However, as in the second trial, the pigs on skimmilk

TABLE 6. RESULTS OF FEEDING SWINE RATIONS CONTAINING EITHER DRIED SKIMMILK, CORN OIL MEAL, TANKAGE, OR CORN GLUTEN MEAL AS THE SOLE SOURCE OF PROTEIN

	Lot numbers and treatments			
	1 Skim-milk	2 Corn oil meal	3 Tankage	4 Corn gluten meal
	percent	percent	percent	percent
No. of pigs	4	4	4	4
No. of days	56	56	56	56
Av. initial wt., lb.	46.2	46.2	46.3	46.0
Av. final wt., lb.	134.0	120.0	94.8	79.2
Av. gain, lb.[1]	87.8	73.8	48.5	33.2
Av. daily gain, lb.	1.57	1.32	0.87	0.59
Av. total feed, lb.[2]	201.6	266.8	267.2	242.5
Av. daily feed, lb.	5.39	4.76	4.77	4.33
Feed per cwt. gain, lb.	344	362	551	729
Protein efficiency[3]	2.64	2.51	1.65	1.25

Least significant difference for P=5% and 1% respectively for the following footnotes was.
[1] 13.5 and 19.0 lb.
[2] 30.3 and 42.4 lb.
[3] 0.27 and 0.38 lb.

grew significantly faster than those receiving corn oil meal. In spite of this difference in rate of growth, there was practically no difference in protein efficiency between these two sources of protein.

Although tankage was inferior in protein quality as measured by pig gains, it was a better source of protein than corn gluten meal. Both tankage and corn gluten meal were decidedly poorer in protein quality as measured by pig gains and protein efficiency than either skimmilk or corn oil meal.

Discussion

The results obtained in this series of feeding trials using semi-purified rations would seem to indicate that the protein efficiency technique is a satisfactory procedure for evaluating natural sources of protein for young growing pigs. In using the protein efficiency technique it is

necessary to make the non-protein fraction of the ration nutritionally complete and allow the source of protein to be the only variable. Only by taking care of all of the required nutrients except protein, may the plant protein feeds be compared on the same basis for protein quality with the animal protein feeds.

It should be pointed out that the results of the experiments presented here do not show necessarily the value of the various protein rich feeds as supplements to grain or their value in supplying nutrients other than protein to the ration. For instance, tankage might show up considerably better as a supplement to corn where it would be contributing minerals and vitamin factors besides supplementary protein at a higher total level.

In the last two trials, the incorporation of a commercial vitamin B_{12} concentrate has supplied only about one unit of protein for every 750 units of test protein. This represents considerable refinement over the procedure as used in the first trial where dried whole liver was supplying one unit of protein for every 21 units of test protein.

The protein in corn steep water solubles was shown to be of such low value that pigs receiving it were unable to maintain body weight. Although no previous reports on corn solubles protein for swine are available, the work by Schulz and Thomas (1949) has shown the biological value of corn solubles for rats to be very low.

Corn oil meal compared very favorably with several of the leading protein feeds in this series of tests. The protein of corn oil meal was only slightly less efficient for growth than whole egg protein and was nearly equal in quality to skimmilk protein. In other protein efficiency studies using rats as the test animals, the protein of the corn embryo has supported efficiency values just below egg (Beeson *et al.*, 1947) and dried skimmilk (Hove *et al.*, 1945; Jones and Widness, 1946).

It is apparent from the data reported in these trials that the consumption of the corn oil meal ration was consistently lower than any of the other adequate rations. The high total crude fiber content of corn oil meal was eliminated as a factor related to palatability since the fiber content of all of the test rations was equalized. Skinner and Star (1918) blamed unpalatability as the cause of low consumption by swine of rations containing corn oil meal. Later work by Smith (1947) shows that corn oil meal has an inherent high water-absorbing capacity which makes the animal become full on less total dry feed.

Corn gluten meal was fed in only one trial in this experiment. The results indicate that the protein of corn gluten meal is inadequate as

the sole source of protein in the ration for swine. Not only did corn gluten meal support very slow pig growth but also the protein efficiency value was only about one-half the value of that for either skimmilk or corn oil meal. In refined nitrogen balance tests with rats (Olson and Palmer, 1940) and with chicks (Van Landingham *et al.*, 1942), it was found that proteins of corn gluten meal were of low quality.

In view of the rather rapid growth made by several lots of young weanling pigs in this series of tests when receiving only 11% protein in the ration, it seems that our present recommended levels of protein for swine (National Research Council, 1944) can be decreased considerably in the future by (1) paying particular attention to protein quality and (2) making the non-protein fraction of the ration nutritionally complete.

Summary

The quality of protein in corn oil meal, corn steep water solubles, soybean oil meal, tankage, and corn gluten meal was evaluated with pigs by using semi-purified rations containing 11% of a single source protein. Dried whole egg and dried skimmilk served as the source of protein in the control rations. The protein efficiency was expressed as pounds gained per pound of protein consumed.

In the first experiment, dried whole egg supported faster pig growth and returned a higher protein efficiency than corn oil meal. The pigs receiving corn solubles lost weight and could not survive without an additional source of protein.

Dried skimmilk supported the most rapid pig growth in the second experiment. Corn oil meal and soybean oil meal were intermediate as far as growth was concerned, and the combination of equal parts of corn solubles and soybean meal resulted in slow growth. Corn oil meal showed the highest protein efficiency followed in order by dried skimmilk, soybean oil meal, and the soybean oil meal-corn solubles mix.

In the last experiment there was a significant difference in growth rate between all lots. Dried skimmilk was superior, followed by corn oil meal, tankage and corn gluten meal in that order. The quality of protein as measured by protein efficiency was highest for skimmilk. Corn oil meal, tankage and corn gluten meal followed in that order.

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EFFECT OF NICOTINIC ACID, VITAMIN B_{12} AND AUREOMYCIN ON GROWTH OF PIGS AND ON RESISTANCE TO ARTIFICIAL INFECTION WITH SALMONELLA CHOLERAESUIS

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THE relationship of diet to necrotic enteritis in young pigs has been under investigation in the Bureau of Animal Industry for several years. Some of the results dealing with nicotinic acid deficiency and the effects of nicotinic acid and of tryptophane, when added to diets deficient in these factors, have been reported by Powick *et al.* (1947, 1948a, 1948b). A separate phase of these experiments, thus far unreported, dealt with the effects upon the experimental animals of artificial infection with Salmonella choleraesuis. The well known antibiotic properties of aureomycin, together with its more recently reported growth stimulating effect upon young pigs (Carpenter, 1950 and Jukes *et al.*, 1950) suggested the following experiment designed to test the relative effects of aureomycin, vitamin B_{12} and nicotinic acid and their combinations, upon growth and upon resistance to artificial infection with Salmonella choleraesuis.

Experimental

The experiment was set up as a 2^3 factorial type which, with an economy of animals, permits a statistical evaluation of the effects of all possible combinations of the three factors, all animals being used in the estimate of error. Thirty-two pigs were divided into eight groups of four pigs each, balanced as to litter and weight. The pigs varied from 59 to 62 days of age at the beginning of the experiment and averaged 12.5 kg. in weight. All were in excellent condition.

A basal diet formulated as follows was prepared:

Casein (Sheffield New Process)	12.23 kg.
Ground corn	24.00 kg.
Ground whole oats	21.55 kg.
Dextrin	30.70 kg.
Lard	7.70 kg.
Mineral mixture	3.50 kg.

[1] The authors wish to express their appreciation of the assistance rendered by Mr. Paul Kerns in the mixing of diets and the supervision of the experimental animals.

The mineral mixture included adequate amounts of trace elements including cobalt. Also included in the basal diet were adequate supplements of thiamin, riboflavin, pyridoxine, pantothenic acid, A and D feeding oil, and alpha-tocopherol. As fed, the diet contained 15% of crude protein. Analyses indicated the presence of amino acids and vitamins as follows:

Arginine	0.55%
Histidine	0.40%
Isoleucine	1.00%
Leucine	1.30%
Lysine	1.02%
Methionine	0.56%
Phenylalanine	0.80%
Threonine	0.64%
Valine	0.91%
Nicotinic acid	1.02 mg. per 100 gm.
Vitamin B_{12}	0.15 mg. per 100 gm.

As calculated from values in the literature, the diet also contained 0.17% of tryptophane.

This diet was modified for the several experimental groups of animals by appropriate additions of nicotinic acid, vitamin B_{12} concentrate (a fermentation product without antibiotic activity), and aureomycin according to the following factorial schedule for groups A to H:

	A	B	C	D	E	F	G	H
Nicotinic acid, mg./100 gm.	0	4	0	4	0	4	0	4
Vitamin B_{12} mmg./100 gm.	0	0	2.32	2.32	0	0	2.32	2.32
Aureomycin, mg./100 gm.	0	0	0	0	4.4	4.4	4.4	4.4

Throughout the experiment the eight diets were fed to the respective groups at the daily rate of 5% of the last observed weight of the animals, except when lesser amounts were indicated because of impaired appetite.

The pigs of each group were penned and fed collectively on concrete. No bedding was used and the pens were washed frequently. The pigs were weighed individually at weekly intervals; and the percentage of total gain over the initial weight, and the percentage of gain within the preceding week were calculated for each pig.

After a period of six weeks on this schedule, each surviving pig received, mixed with its feed, a standard dose of a 24-hour broth culture of Salmonella choleraesuis, and without change in dietary treatment was held under observation for three additional weeks.

Results and Discussion

The mean gains for the four pigs in each group, calculated as indicated above, are presented in table 1. If groups A, B, E and F, which received no vitamin B_{12}, are compared respectively with groups C, D, G and H, which received corresponding treatments except for the addition of this factor, one is immediately impressed by the absence of any considerable differences. Statistical analysis confirms the impression that the addition of vitamin B_{12} to the basal diet was without significant effect.

TABLE 1. MEAN PERCENTAGE OF GAIN AT WEEKLY INTERVALS OF PIGS RECEIVING BASAL DIET PLUS INDICATED ADDITIONS OF AUREOMYCIN, VITAMIN B_{12} AND NICOTINIC ACID

Week of experiment	No Aureomycin				Aureomycin Added			
	No B_{12} added		B_{12} added		No B_{12} added		B_{12} added	
	No NA[1] added % gain	NA added % gain	No NA added % gain	NA added % gain	No NA added % gain	NA added % gain	No NA added % gain	NA added % gain
	From data on percentage of cumulative gains							
2	13.5	30.2	21.1	30.4	39.7	34.5	31.3	35.7
3	11.0	54.7	18.8	55.5	60.9	59.4	57.5	63.4
4	12.1	91.8	19.5	85.9	63.0	88.1	63.0	102.0
5	5.1	121.8	16.7	112.6	60.2	120.0	62.8	129.8
6	0	159.9	21.2	146.3	64.1	150.7	67.9	164.5
	From data on percentage of gains within successive weeks							
2	2.7	20.6	13.1	19.8	22.2	22.3	19.3	24.0
3	—2.3	18.8	—1.7	19.2	15.2	18.4	19.9	20.4
4	1.6	23.8	0.8	19.6	1.1	18.3	3.2	23.7
5	—6.4	15.6	—2.4	14.3	—1.7	16.8	0.1	13.8
6	—5.3	17.1	3.8	15.7	2.2	14.2	2.6	15.1

[1] NA=Nicotinic acid.

Highly significant effects, however, were observed from the addition of nicotinic acid and of aureomycin. These effects are indicated in table 2, which shows the mean percentage of gain of the pigs in the several categories, the differences in gain attributable to diet, and the significance of these differences as indicated by an analysis of variance.

It will be observed that by the end of the second week of the experiment the nicotinic acid had produced a highly significant acceleration of growth in the pigs receiving no aureomycin, an effect that was main-

TABLE 2. MEAN GAINS PERCENT AT WEEKLY INTERVALS AND EFFECTS OF NICOTINIC ACID AND AUREOMYCIN ON MEAN GAINS

Week of experiment	Mean gains of pigs receiving basal diet plus indicated additions				NA effect			AU effect			Interaction NA X AU
	No AU, no NA % gain	No AU, NA added % gain	AU added, No NA % gain	AU added NA added % gain	AU not added	AU added	Mean effect	NA not added	NA added	Mean effect	
	From Data on Cumulative Percentage of Gains at End of Successive Weeks										
2	17.3	30.3	35.5	35.1	13.0**	—0.4	6.3*	18.2**	4.8	11.5**	—13.4*
3	14.9	55.1	59.2	61.4	40.2**	2.2	21.2**	44.3**	6.3	25.3**	—38.0**
4	15.8	88.8	63.0	95.0	73.0**	32.0**	52.5**	47.2**	6.2	26.7**	—41.0**
5	10.9	117.2	61.5	124.9	106.3**	63.4**	84.8**	50.6**	7.7	29.2**	—42.9**
6	10.6	153.1	66.0	157.6	142.5**	91.6**	117.0**	55.4**	4.5	30.0**	—50.9**
	From Data on Percentage of Gains Within Successive Weeks										
2	7.9	20.2	20.8	23.2	12.3**	2.4	7.4**	12.9**	3.0	7.9**	—9.9**
3	—2.0	19.0	17.6	19.4	21.0**	1.8	11.4**	19.6**	.4	10.0**	—19.2**
4	1.2	21.7	2.1	21.0	20.5**	18.9**	19.7**	.9	—.7	.1	—1.6
5	—4.4	15.0	—.8	15.3	19.4**	16.1**	17.8**	3.6	.3	2.0	—3.3
6	—.7	16.4	2.4	14.7	17.1**	12.3**	14.7**	3.1	—1.7	.7	—4.8

NOTE.
* P=5%
** P=1%
NA=Nicotinic acid
AU=Aureomycin

tained within each successive week of the nutritional phase of the experiment.

During the first three weeks of the experiment, the aureomycin, in the absence of nicotinic acid, was about as effective as the nicotinic acid in the absence of aureomycin in producing a highly significant acceleration in growth. But whereas the effect of this initial stimulus by aureomycin was maintained to the end of the sixth week, the aureomycin seemed to have no further effect during the last three weeks of the nutritional phase.

Among the pigs receiving aureomycin, the addition of nicotinic acid appeared to be without effect during the first three weeks of the experiment. It seemed as if the aureomycin itself was providing the pigs' requirement for nicotinic acid. From the third week on, however, as the aureomycin effect disappeared, growth was retarded in the pigs receiving no nicotinic acid, and the advantage of nicotinic acid addition became highly significant during the second three weeks.

Finally, in the presence of adequate nicotinic acid, no effect was produced at any time by the further addition of aureomycin.

The mean nicotinic acid effects and the mean aureomycin effects shown in the table are simply averages of the particular effects of these supplements discussed above. The interaction column represents the extent to which the aureomycin and the nicotinic acid were each able to obscure the effect of the other, i.e., to replace the other.

During the post-infection period and at necropsy, no particular differences were noted between pigs receiving aureomycin and corresponding pigs receiving none, with respect to temperature elevation, loss or gain in wight, agglutinins in blood, Salmonella recovered from tissue samples taken at necropsy, or necrotic lesions in the large intestine. The same applied to vitamin B_{12} and nicotinic acid, except that the groups receiving nicotinic acid continued to gain in this period of the experiment, while the groups deficient in nicotinic acid lost in weight. Except in group A, there was little evidence of necrotic enteritis in this experiment.

The nicotinic acid effects observed in this experiment are neither new nor unexpected, but are in line with those obtained in experiments already reported by ourselves and others.

The absence of vitamin B_{12} effect in this experiment is perhaps worthy of note, although the possibility that the casein directly or the cobalt indirectly supplied part or all of the requirement for this vitamin must not be overlooked. That the pigs could have obtained this factor from their droppings seems unlikely, since with few exceptions, the

pens were washed daily and furthermore Groschke *et al.* (1950) report that pig feces show no vitamin B_{12} activity in the absence of fermentation.

That the aureomycin should be able to cover a nicotinic acid deficiency for a period of three weeks and thereafter become ineffective, is a matter of considerable interest. It is also interesting to speculate as to the mechanism of this curious effect, and the following hypothesisis is suggested:

As has been shown in case of thiamin in yeast, (Melnick *et al.*, 1945; Parsons *et al.*, 1945a and b) it may be postulated that accessory factors contained in live microorganisms within the intestinal tract are, in general, not very available to the host, whereas those in dead organisms become so. The aureomycin, by killing certain types of intestinal bacteria, could thus render their accessory factors available to the host. During the period when this is happening, the nicotinic acid requirement of an animal might be covered by the nicotinic acid released from the killed microorganisms. By the end of three weeks it would seem that only organisms resistant to aureomycin might remain, so that this source of nicotinic acid would be lost to the host.

Assuming such a hypothesis to be valid, it would seem to follow that similar effects might be expected in connection with accessory factors other than nicotinic acid and with antibiotics other than aureomycin. It would also seem that intermittent feeding of aureomycin, with suitably chosen intervals to permit restoration of the original bacterial flora, might permit a repetition of the initial effect.

Summary

In a pig-feeding experiment, the effects of adding nicotinic acid, vitamin B_{12} and aureomycin, separately and in combination, to a diet low in nicotinic acid and in B_{12}, have been reported. The effects observed were as follows:

1. The addition of nicotinic acid resulted in the usual highly significant stimulation of growth.
2. The addition of vitamin B_{12} was without effect.
3. The addition of aureomycin resulted in a highly significant stimulation of growth sharply limited to the first three weeks of the experiment.
4. Feeding of a broth culture of Salmonella choleraesuis at the end of the the sixth week of the experiment produced little effect on any of the pigs and no significant differences in response that could be related to the dietary treatments.

A possible hypothesis to account for the growth-stimulating effect of aureomycin has been suggested.

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SPECIAL SOLVENT COTTONSEED MEAL WITH AND WITHOUT A VITAMIN B_{12} SUPPLEMENT AND STREPTOMYCIN AS A SOURCE OF PROTEIN FOR PIGS[1]

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THE nutritive value of cottonseed meal as a protein supplement for swine has largely been obscured in the past by the toxicity of this product to non-ruminant animals. New methods for the effective detoxification of cottonseed meal, however, give promise of a more extensive use of it in swine rations. Consequently, reliable information on the nutritive value of the detoxified product is needed.

Reports on the nutritional value of "detoxified" cottonseed meal vary greatly. Differences in the level of free gossypol left in a particular sample, components of the ration other than cottonseed meal as well as the species and nutritional background of the animal used appear to be factors contributing to this lack of agreement.

Richardson and Blaylock (1950) reported an unsatisfactory growth of poults when fed a milo-cottonseed meal diet. When 0.3% L lysine was added to this diet, white feathers, perosis and a slow growth rate persisted. However, when 0.8% L lysine was added the performance was satisfactory. Heywang and Bird (1950), working with chickens reported that lysine and probably vitamin B_{12} was inadequate in cottonseed meal. They further indicated that APF was not the first limiting factor in cottonseed meal, but that it was more deficient in a factor(s) found in sardine fish meal. Sherwood and Couch (1950) also reported a satisfactory growth rate when both lysine and APF were added to a chick's ration in which cottonseed meal was used as the protein supplement.

The response of pigs to either vitamin B_{12} supplement or antibiotics has also been quite variable. Rations most nearly balanced are apparently affected less by the addition of vitamin B_{12} than are less well balanced rations. Luecke *et al.* (1950), Cunha (1950) and others

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have indicated that streptomycin, generally, has increased the rate of gain only slightly whereas aureomycin in most instances has given a greater growth response.

The objects of this experiment were to: (1) determine if "detoxified" cottonseed meal could safely be used as the only protein supplement for pigs; (2) study the value of this cottonseed meal as a protein supplement for pigs when fed either with or without a vitamin B_{12} supplement and streptomycin; (3) study its value when fed in combinations with peanut and soybean oil meals and with soybean oil meal alone.

TABLE 1. RATIONS FED FROM WEANING TO 100 POUNDS IN DRYLOT

Item	Lot						
	1	2	3	4	5	6	7
Ground white corn, %	71	63	63	65	65	63	64
"Detoxified" solvent cottonseed meal, %[1]	—	35	35	11	11	35	17
Meat and bone scraps, %	27	—	—	—	—	—	—
Solvent soybean oil meal, %	—	—	—	11	11	—	17
Peanut oil meal, %	—	—	—	11	11	—	—
Mineral mixture, %[2]	2	2	2	2	2	2	2
Streptomycin[3]	—	—	—	—	—	+	—
B_{12} supplement[4]	—	—	+	—	+	+	—
Vitamin additions[5]	+	+	+	+	+	+	+

[1] Contained less than 0.03% free gossypol.

[2] Composed of 2 parts Deflorophos (31% calcium, 13% phosphorous and not more than 0.08% fluorine), 2 parts ground limestone and 1 part salt.

[3] 20 mg. (38.7 percent base content) added per pound of feed.

[4] 12.5 mcg. per pound of ration of Merck's APF supplement (12.5 mg. B_{12} activity per pound) was added.

[5] Vitamin additions per pound of diet consisted of 500 IU A, 90 IU D, 1.5 mg. riboflavin, 5 mg. niacinamide, 6 mg. calcium pantothenate, and 250 mg. choline chloride.

Experimental Procedure

Seven groups of nine weanling Duroc and Poland China pigs each were self-fed a 20% crude protein ration in drylot. Pigs were allotted as equally as practicable on the basis of weight, litter, sex and breed at 56 days of age. Water was supplied for *ad libitum* consumption. The dams of all pigs had been on Ladino clover pasture during gestation and lactation.

The basal ration was composed of ground white corn 71%; meat and bone scraps 27%; and mineral mixture 2%. The protein concentrate for the remaining six lots was made up entirely or in part of cottonseed meal. Rations for all groups were fortified with vitamins A, D, riboflavin, niacin, pantothenic acid and choline. Treatment of the seven groups is presented in table 1.

Results and Discussion

Pigs in lot 1 did not perform satisfactorily. Since such a ration ordinarily supports a much faster rate of gain, it appears obvious that the meat and bone scraps were of poor quality. When cottonseed meal replaced the meat and bone scraps (lot 2) the rate of gain was increased although not significantly. This level of "detoxified" cottonseed meal produced no typical symptoms of gossypol toxicity as was observed earlier by Dyer and Cullison (1949) when commercial cottonseed meal was fed to pigs as 35% of the diet. The further addition of B_{12} supplement did not significantly increase the rate of gain (lot 3). Gains made by pigs in lots 2 and 3, although improved over those produced by the basal ration, were still unsatisfactory. The average daily feed consumed per pig was 0.75 pounds greater when B_{12} was fed. At approximately 100 pounds, dermatitis and a depigmentation of the hair coats occurred in lots 2 and 3. Scrapings from the infected areas of pigs with dermatitis were taken. No mange mites were present. The level of B_{12} fed to pigs in lot 3 had no apparent effect on either dermatitis or depigmentation. This depigmentation might suggest that cottonseed meal supplies a suboptimal amount of lysine for the pig. This observation is corroborated by Heywang and Bird (1950) with chickens. It is likely that these deficiency symptoms would have occurred earlier if the pigs had not had an excellent nutritional background.

When equal mixtures of solvent cottonseed meal, solvent soybean oil meal and peanut oil meal composed the protein supplement the average daily gain was satisfactory and increased ($P<0.001$) over that produced by the basal ration (table 2) and increased ($P<0.05$) over that produced by pigs in either lots 2 or 3. The further addition of vitamin B_{12} supplement only slightly increased the rates of gain (lot 5). No depigmentation or dermatitis were encountered in either lots 4 or 5. The addition of streptomycin to a corn-cottonseed meal ration plus B_{12} supplement increased the rate of gain although not significantly (lot 6). Dermatitis as well as an unthrifty appearance accompanied by a loss in weight was so severe in this lot after they reached approximately 100 pounds liveweight that only 3 pigs finished the experiment. The average daily feed consumed by the remaining pigs was higher in lot 6, as was true also for lots 3 and 5, than the average for pigs not receiving the B_{12} supplement.

An equal mixture of solvent soybean and cottonseed meals as the protein supplement increased the rates of gain ($P<0.001$) over

those produced by the basal ration. This rate of gain (lot 7) was also significantly faster than in any of the other 5 lots.

Pigs in lots 3 and 6 (affected with dermatitis and already receiving vitamin B_{12} supplement) were injected intramuscularly with 10 mcg. of crystalline B_{12} weekly for 6 weeks. This increased level of B_{12}, initially, appeared to partially alleviate the dermatitis. As will be noted by the number of pigs removed from lots 3 and 6 (table 2) B_{12} was apparently not the chief factor concerned with dermatitis. Dermatitic pigs removed from this test and placed on Ladino clover pasture recovered completely in from 15–30 days.

TABLE 2. EFFECT OF "DETOXIFIED" COTTONSEED MEAL, B_{12} SUPPLEMENT, STREPTOMYCIN, SOLVENT SOYBEAN OIL MEAL AND PEANUT OIL MEAL ON THE GROWTH RATE AND FEED EFFICIENCY OF PIGS

	Lot[1]						
	1	2	3	4	5	6	7
No. of pigs started	9	9	9	9	9	9	9
Av. initial weight, lb.[2]	34.1	34.0	34.2	34.1	34.0	34.4	35.4
No. pigs that finished test[3]	8	4	6	8	8	3	8
Av. final weight, lb.[4]	185.6	186.5	202.0	202.9	195.9	169.3	208.1
Daily gain, lbs.							
Weaning to 100 lbs.. Avg.	.68	.84	.91	1.23**	1.34**	1.33**	1.61**
S.E.	.07	.15	.13	.07	.08	.07	.08
Weaning to 200 lbs., Avg.	1.00	1.19	1.13	1.44**	1.54**	1.29	1.75**
S.E.	.05	.13	.09	.04	.06	.09	.05
Av. daily feed/pig, lb.	4.15	5.05	5.80	5.05	5.83	5.95	5.96
Feed eaten/100 lbs. gain to final weight, lb.	406.	417.	460.	355.	379.	448.	347.

** Gained significantly faster ($P<0.001$) than pigs in lot 1.

[1] See table 1 for rations fed.

[2] Average initial weight of pigs started on test.

[3] One pig in each of lots 4, 5 and 7 removed because of rectal prolapse; 1 pig in lot 1 removed because of abdominal hernia and unthriftness; pigs were removed from lots 2, 3 and 6 with severe cases of dermatitis.

[4] Average final weight of pigs that finished test.

Summary and Conclusions

As high as 35% of the total ration was fed as "detoxified" cotton seed meal to weanling pigs. No typical symptoms of gossypol toxicity occurred. However, dermatitis, depigmentation of the hair coat of the Duroc pigs, a slow rate of growth as well as a reduction in feed efficiency were encountered. These deficiency symptoms suggest that this "detoxified" cottonseed meal is deficient in factors essential for optimum growth and physiological well-being of the pig. Results of this test indicate that neither vitamin B_{12} nor streptomycin are the first limiting factors. When cottonseed meal and solvent soybean oil

meal were fed together (each comprising 50% of the protein supplement), the soybean oil meal apparently supplied some factor(s) in which cottonseed meal was deficient.

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ANIMAL PROTEIN FACTOR AND ANTIBIOTIC SUPPLEMENTATION OF SMALL GRAIN RATIONS FOR SWINE[1]

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STUDIES on the addition of APF and vitamin B_{12} supplements to swine rations have been reported by a number of workers during the past two years. Among the authors whose results showed increased growth rate in pigs when APF supplements were added to swine rations were Burnside *et al.* (1949); Cunha *et al.* (1949, 1950); and Luecke *et al.* (1949). From the data obtained it was realized that in these trials the APF contained some growth promoting factor(s) other than vitamin B_{12}. However, it was some time before the Florida and Lederle Laboratory workers jointly showed that an antibiotic in the APF supplement was at least one of these factors. Jukes *et al.* (1950) found that the addition of aureomycin and vitamin B_{12} to rations of weanling pigs produced greater gains than vitamin B_{12} alone.

The study of the effect of antibiotics in swine rations has gained momentum rapidly since it was shown that they are growth promoting substances. Cunha *et al.* (1950a) reported that APF (Lederle) is concerned with efficiency of feed utilization, rate of gain and hemoglobin levels, while Lepley *et al.* (1950) found that the addition of whole aureomycin mash increased average daily gains significantly. The majority of the work reported to date has been carried out using a basal corn ration or a purified diet with little work reported on the addition to small grain rations of APF supplements with or without antibiotics.

Experimental

Experiments were initiated by the authors in the fall of 1949 to study APF supplementation of typical western Canadian rations containing either all vegetable or mixed animal-vegetable protein. The

[1] Acknowledgement is made to Mr. H. Johannson, Merck and Co. Ltd., Montreal, for supplying Merck APF-3, and to Dr. T. H. Jukes, Lederle Laboratories, Pearl River, New York, for supplying Lederle APF-5. Representatives of the Production Service, Canadian Department of Agriculture, co-operated in carrying out the cutting and scoring of carcasses by Advanced Registry standards.

[2] Experiments 2, 3 and 4 were conducted in partial fulfillment of the requirements for the degree of Master of Science.

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first object of these experiments was to study the effect on rate and economy of gain and on carcass quality in bacon-type hogs when basal barley-oat rations were fortified with an APF supplement containing no antibiotic or with one containing aureomycin. Two sources of supplement were used; APF-3 (Merck) containing 12.5 mg. of vitamin B_{12} per lb., and APF-5 (Lederle) containing residual aureomycin in addition to vitamin B_{12}. A second object was to study the effect of the addition of these APF supplements to two rations containing different protein supplements; one of all vegetable origin and the other of mixed animal-vegetable origin. Because of the results obtained with thrifty pigs a further series of experiments was run to study the effect of APF plus antibiotic when it was added to the rations of unthrifty or "runty" weanling pigs. Throughout the remainder of the paper APF-3 (Merck) will be referred to as APF, and APF-5 (Lederle) will be referred to as APF+.

Purebred Yorkshire weanling pigs from the University herd were allotted uniformly on the basis of litter, sex, weight, thriftiness and age in all experiments. In experiments 1 and 2 where carcass studies were made, distribution as to lot was based also on type. All experiments were conducted in drylot on concrete floored pens with raised wooden sleeping floors. Water was supplied *ad libitum*.

The basal rations fed are shown in table 1.

It will be noted that the protein level in all rations was reduced when the pigs averaged 125 lb. in weight. The barley-oat ratio was altered during the growing period in experiments 2, 3 and 4 compared to experiment 1. In these latter experiments the protein supplement in the all vegetable protein ration was lowered to make the ration approximately equal in protein level to the mixed animal-vegetable protein ration.

Experiment 1

Four uniform lots of seven 30-pound weanling Yorkshire pigs each were hand-fed to final weights of 200 pounds. Lot 1 received an animal-vegetable protein ration (ration 1) as shown in table 1; lot 1A received ration 1 plus 50 gm. of APF per 100 lb. of feed; lot 2 received an all vegetable protein ration (ration 2); while lot 2A received ration 2 plus 50 gm. of APF per 100 lb. of feed.

Experiment 2

Six uniform lots of seven 30-pound weanling Yorkshire pigs each were self-fed to final weights of 200 pounds. Lot 1 received an animal-

vegetable protein ration (basal ration 3) as shown in table 1; lot 1A received ration 3 plus 50 gm. of APF per 100 lb. of feed; lot 1B received ration 3 plus 0.5% of APF+; lot 2 received an all vegetable protein ration (basal ration 4); lot 2A received ration 4 plus 50 gm. of APF per 100 lb. feed; lot 2B received ration 4 plus 0.5% APF+.

TABLE 1. BASAL RATION COMPOSITION

Item	Experiment 1		Experiments 2, 3 & 4	
Ration No.	1	2	3	4
Ration	Animal-Vegetable Protein	Vegetable Protein	Animal-Vegetable Protein	Vegetable Protein
30–125 lb.[1]				
Barley	53	51	63	63
Oats	35	34	25	25
Supplement[2]	12	15	12	12
Protein, %	13.9	15.1	13.5	12.8
125–200 lb.				
Barley	58	55.5	58	58
Oats	36	37	36	36
Supplement[2]	6	7.5	6	6
Protein, %	12.5	12.0	11.6	11.4

[1] 10,000 I.U. vitamin A and 1,667 I.U. vitamin D per pig daily were fed in the form of 1200A-200D feeding oil from 30 to 125 lb. in weight.

[2] Supplements were as follows:

	Animal-Vegetable Protein	Vegetable Protein
Meat scrap	50	—
Soybean oil meal	—	50
Linseed oil meal	25	25
Alfalfa meal	15	15
Iodized salt	5	5
Limestone	5	5
	100 lb.	100 lb.

All pigs in experiments 1 and 2 were weighed individually every second week and more frequently when they approached market weight. Carcass data were obtained in these experiments.

Experiments 3 and 4

Two uniform lots of 7 unthrifty pigs each were used in each of these experiments. In experiment 3 the initial weight of the pigs in each lot averaged approximately 25 lb. while in experiment 4 it

averaged approximately 22 pounds. Lot 1 in each case received the all vegetable protein ration 4 shown in table 1, whereas lot 2 received this ration supplemented with 0.5% APF+. All pigs were weighed individually every week. Hemoglobin determinations were run on the blood of the pigs according to the method of Evelyn (1936) using an Evelyn photoelectric colorimeter.

Results and Discussion

Experiment 1

A summary of experiment 1 is given in table 2.

TABLE 2. APF SUPPLEMENTATION OF ANIMAL-VEGETABLE OR ALL VEGETABLE PROTEIN RATIONS

	Lot			
Item	1	1A	2	2A
Ration	Basal 1[2]	Basal 1[2] & APF	Basal 2[3]	Basal 2[3] & APF
No. of pigs in lot	7	7	7	7
Av. no. days fed[1]	146	143	154	140
Av. initial weight[1] lb.	30	27	27	27
Av. final weight[1] lb.	199	201	201	201
Av. daily gain lb.	1.15	1.22	1.13	1.25
Av. daily feed lb.	4.87	4.94	4.57	4.92
Feed/100 lb. gain[1] lb.	422	405	403	394
Av. dressing % age (hot)[1]	76	76	75	76
Carcass grades: A	4	4	4	1
B_1	3	3	3	6
C	0	0	0	0

[1] Figures, in this and subsequent tables, are rounded to the nearest whole number.
[2] Animal-vegetable source of protein.
[3] Vegetable source of protein.

The pigs in lots 1A and 2A receiving APF supplement gained slightly more rapidly and efficiently than those in the control lots. However, these differences were not statistically significant. There was no difference in average daily gain due to source of protein. However, as the all-vegetable protein ration fed to lots 2 and 2A during the growing period contained 1.2% more protein than the mixed animal-vegetable protein ration for lots 1 and 1A, the average daily gains may not be strictly comparable. The pigs in the all vegetable protein lots showed a trend toward more efficient gains than those in the mixed protein lots. Pigs in group 2A, fed an all vegetable ration supplemented with APF ate 0.35 lb. more feed per day than

their controls in group 2, but there was no difference in feed consumption between the supplemented and unsupplemented pigs receiving a mixed animal-vegetable protein ration.

Experiment 2

Experiment 2 was similar to experiment 1 except that two additional lots were added receiving APF containing residual aureomycin (APF+). To allow the APF supplements to have their full effect, if this is due partly to increased feed consumption, the pigs in this experiment were self-fed. The protein level of the ration for the

TABLE 3. APF AND APF PLUS ANTIBIOTIC SUPPLEMENTATION OF ANIMAL-VEGETABLE OR ALL VEGETABLE PROTEIN RATIONS.[1]

Item	Lot					
	1	1A	1B	2	2A	2B
Ration	Basal 3[2]	Basal 3[2] & APF	Basal 3[2] & APF+	Basal 4[3]	Basal 4[3] & APF	Basal 4[3] & APF+
No. pigs in lot	7	7	7	7	7	7
Av. no. days fed	134	132	114	143	133	117
Av. initial weight lb.	31	31	31	31	30	31
Av. final weight lb.	199	202	202	201	199	201
Av. daily gain lb.	1.26	1.29	1.51**	1.18	1.27*	1.46**
Av. daily feed lb.	5.10	5.02	5.66	4.49	4.75	5.20
Feed/100 lb. gain lb.	405	388	376	380	375	357
Av. dressing %age (hot) %	77	77	78	77	75	78
Carcass Grades: A	1	2	0	2	2	0
B_1	6	4	6	5	5	6
C	0	1	1	0	0	1
Av. A. R. Score	67	62	57	69	63	53

*P<0.05 significant by analysis of variance, Snedecor (1946).
** P<0.01 highly significant by analysis of variance.
[1] Lederle APF fed was from Experimental Lot No. 26.
[2] Animal-vegetable source of protein.
[3] Vegetable source of protein.

vegetable protein lots was lowered from the former experiment to bring it more nearly into line with the animal-vegetable protein lots.

A summary of experiment 2 is given in table 3.

The pigs in the lots receiving APF supplement showed increased average daily gains and feed efficiency over the pigs in the control lots. The average daily gains of the APF supplemented pigs receiving all vegetable protein were significantly higher than those of the controls, but in the case of those on the mixed animal-vegetable protein the increase in average daily gains was not significant. It would appear that the APF tended to equalize the two rations regardless of protein source.

The APF+ supplement caused a highly significant increase in average daily gains over either of the control rations. This APF supplement with antibiotic activity gave a significant increase in average daily gains over and above that brought about by the APF supplement without antibiotic. Average daily feed consumption of the pigs was increased greatly in the lots receiving APF+ supplement but the feed efficiency of the supplemented pigs was superior to that of those receiving APF.

The effect of the APF+ supplement was greatest during the growing period when average daily gains were increased 24% and feed efficiency was increased 9 percent. The comparable figures for the growing-fattening period were 15 and 5 percent. In both experiments 1 and 2 the response to APF of the pigs receiving mixed animal-vegetable protein differed very little between the growing and the growing-fattening periods. In no case was there a significant difference in average daily gain or in feed efficiency between the pigs in the mixed protein control lots and those in the APF supplemented lots. However, in both experiments the pigs in lot 2A receiving APF supplement with an all vegetable protein ration exceeded the pigs in the control lots in rate and efficiency of gain to a greater extent during the growing-fattening period than during the growing period. A summary of the data from both experiments indicated an increase over the pigs in the control lots of 5% in average daily gains and a decrease of 2% in feed efficiency up to 125 lb., compared to an increase of 14% in average daily gains and 6% in feed efficiency during the period from 125 to 200 pounds. This would indicate that the APF supplement exercised greatest effect after the protein content was lowered when the rations were changed at an average weight of 125 pounds. As in the previous experiment, the pigs in the all vegetable protein lots made more efficient gains than the pigs in the mixed animal-vegetable protein lots.

Luecke *et al.* (1949) and Lepley *et al.* (1950) did not find a difference in feed utilization between pigs receiving a supplement of APF and those on a control corn-soybean oil meal ration. However, Cunha *et al.* (1950a) reported increased feed efficiency when APF was added to a corn-peanut meal ration, which is in agreement with the results obtained in the trials reported in this paper with pigs on a barley-oat basal ration supplemented with either soybean oil meal and linseed oil meal, or meat scrap and linseed oil meal.

The effect of APF supplements on carcass quality is of considerable economic importance because of the Canadian production of a bacon-

type hog. As shown in table 3, there was a trend for APF with antibiotic to increase dressing percentage and lower carcass grades (graded by government graders under standards of the Dominion Government Hog Carcass Grading Policy). The carcass grading data in tables 2 and 3 indicate no definite effect of APF alone. On the basis of Advanced Registry Score, which is based on exact measurements and therefore is a more critical criterion of carcass quality, there was a definite trend to lower carcass scores when APF was fed. The decrease in score was greater with APF+ than with APF. Although not statistically significant in any case, the lower score of the pigs in lots 1B and 2B receiving APF+ approached significance at the 0.05 level. Advanced Registry Score measures length of side, evenness and thickness of back fat, balance of side, belly grade, area of loin muscle and type. The principal effect of the APF+ was a reduction in carcass length and an increase in back fat. It would appear that this deleterious effect may be due to very fast gains during the finishing period. McMeekan (1940) has found that bacon-type pigs that are fed to make rapid gains in this period tend to lay down more fat. These pigs on a high plane of nutrition were also shown by McMeekan to have 10% less bone and 17% less muscle than pigs on a low plane of nutrition. It therefore seems logical that skeletal growth and length of side would fail to keep pace with a rapid increase in weight during the finishing period. The live pigs in lots 1B and 2B appeared to be fatter than the controls when they were observed during the fattening period.

Experiments 3 and 4

Because of the immediate response obtained when APF+ was fed to thrifty weanling pigs it was decided to study its effect on unthrifty or "runty" weanling pigs. Table 4 indicates the results with 2 groups of unthrifty pigs which were self-fed.

During the 40 days on trial the pigs in experiment 3 receiving the APF with aureomycin gained 51% faster and made 14% more efficient use of their feed. When the experiment was terminated the pigs were all placed on pasture and received the basal ration without APF+. At the end of 8 weeks the original APF+ lot maintained their lead in weight but failed to gain any faster than the original controls. Average weight after this period on pasture of the pigs in the original APF+ lot was 117 lb. compared to 104 lb. for the pigs in the original control lot. Apparently there was no carryover effect insofar as it affected average daily gains.

In experiment 4 the response of the unthrifty weanling pigs to APF+ was again remarkable. There was an increase of 86% in gains and 20% in feed efficiency when 0.5 percent of the APF+ was fed. At the end of 48 days the rations were reversed with lot 2 now becoming the control. At the end of the first week the rate of gain in the two lots had reversed. However, the original control lot did not catch up to the original APF+ supplemented lot after the reversal of feeds.

Hemoglobin determinations were run on all pigs at the initiation

TABLE 4. EFFECT OF APF SUPPLEMENT CONTAINING AUREOMYCIN ON UNTHRIFTY PIGS[1]

Experiment No.	3		4			
Period			A		B (Reversal)	
Lot No.	1	2	1	2	1	2
Ration	Basal 4	Basal 4 & APF+	Basal 4	Basal 4 & APF+	Basal 4 & APF+	Basal 4
No. of pigs in lot....	7	7	7	7	7	7
No. of days fed.....	40	40	48	48	43	43
Av. initial weight..lb.	25	25	22	22	46	68
Av. final weight...lb.	47	58	46	68	114	131
Av. daily gain....lb.	0.55	0.83**	0.51	0.95**	1.57	1.47
Av. daily feed.....lb.	1.83	2.34	1.79	2.68	5.28	5.39
Feed/100 lb. gain..lb.	330	283	354	282	336	368

** $P<0.01$ highly significant by analysis of variance.
[1] Lederle APF fed was from Lot No. 8108–172.

of each experiment and again at the termination of experiments 3 and 4, and after 8 weeks in experiments 1 and 2. Hemoglobin levels of the blood of the pigs were not found to vary appreciably in any of the experiments, and all appeared to be in the normal range. This was true of the unthrifty pigs in experiments 3 and 4 as well as of the pigs in the earlier trials. Examples of hemoglobin levels may be illustrated by data taken from experiment 3 at the end of the 40-day trial. The average hemoglobin level of the blood of the pigs in the control lot was 9.3 mg. % as compared to 9.2 mg. % for the APF+ supplemented lot. This contrasts with the report of Cunha *et al.* (1950a) where APF+ was found to increase hemoglobin levels.

Summary

Data are presented on the results of experiments with Yorkshire swine fed basal barley-oat rations supplemented with either all vegetable or mixed animal-vegetable protein.

Pigs receiving an APF supplement without antibiotic gained slightly more rapidly and efficiently than the controls but the difference was generally not significant. The amount of response was somewhat greater on an all vegetable protein ration than on an animal-vegetable protein ration.

An APF supplement containing residual aureomycin caused marked increases in rate and efficiency of gain when added to basal rations containing protein of either vegetable or mixed animal-vegetable origin. Unthrifty weanling pigs gave a very great response in increased rate and efficiency of gains to this APF supplement.

There appeared to be a reduction in carcass quality of bacon-type hogs receiving APF supplementation throughout the growing and fattening period. This was particularly noticeable in those receiving a ration supplemented with APF containing aureomycin.

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CARCASS STUDIES ON HOGS OF DIFFERENT BREEDING

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RESEARCH relating to the development and use of inbred lines of swine makes it desirable to evaluate and compare the carcass merit of the lines and their crosses. Can inbred lines be characterized so as to predict merit of the carcasses from crosses between these lines?

Hankins and Hiner (1937) reported characteristic differences in yields of ham and loin in carcasses of the Danish Landrace, Duroc, and Poland China breeds. Winters and co-workers (1948) reported that carcasses from inbred Poland China lines, outbred Poland Chinas, Durocs, and Chester Whites were inferior to those from Poland China line crosses. Crosses of lines of different breeds produced the best carcasses. Minnesota #1, Minnesota #2, and inbred lines of Poland Chinas each transmitted certain different, desirable carcass characteristics in crossing (Sierk, 1949). From the correlation between paternal half-sibs Dickerson (1947) gave estimates of heritability for carcass traits as less than one-third for lean cuts, about one-half for the measure of fatness, and nearly three-fourths for length of carcass.

In light of these findings it was deemed advisable to attempt to characterize certain Duroc inbred lines and to determine if these line characteristics are noticeable in two-line and three-line crosses within the Duroc breed. The effect on carcass merit of crossing the Durocs with two other breeds was also studied.

Materials and Methods

The 136 carcasses used in this study were from the Swine Breeding Project conducted by the Oklahoma Experiment Station in cooperation with the Regional Swine Breeding Laboratory. Four groups of hogs were included in the study, but, because they were handled differently, they cannot be compared directly. Groups one and two were from pigs farrowed in the spring of 1949 and groups three and four were from pigs farrowed in the fall of 1949.

Group one carcasses were from four Duroc two-line cross lots and an outbred Duroc check lot. The carcasses were from the first two

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barrows to reach market weight in each of three litters representing each lot. The pigs were self fed in dry lot and made an average daily gain of 1.39 pounds from weaning (56 days) to slaughter.

Group two pigs were self fed on pasture and gained slower than did the pigs in group one. The average daily gain was 1.19 pounds from weaning to slaughter. These pigs were representatives of inbred Duroc lines 5, C, S, T, and 3 and were nearly all barrows. Gilts from this group were used in the slaughter test only when barrows were not available.

Group three carcasses were from pigs on a fat utilization study and included eight carcasses from each of two Duroc two-line cross lots, a Minnesota #2 x inbred Duroc crossbred lot, and an outbred Duroc lot. These barrows were individually fed and made average daily gains of 1.84 pounds from an initial weight of 53 pounds to a final weight of 230 pounds.

Group four consisted of 53 carcasses, approximately half barrows and half gilts, and included from four to eight representatives of each of two two-line cross Durocs, three three-line cross Durocs, an outbred Duroc lot, one lot each of outbred Poland x line cross Duroc and Minnesota #2 x line cross Duroc crossbreds, and a lot of Landrace-Polands. These pigs were self fed in dry lot and varied considerably in average daily gain from a low of 1.21 for outbred Durocs to a high 1.68 pounds for Cx3–5, three-line cross. The group gained an average of 1.47 pounds per day after weaning.

When the pigs reached the weight range of 219 to 232 pounds, they were taken off feed for twenty-four hours and then slaughtered in the college meat laboratory. Carcasses were prepared packer style with head off and leaf fat removed. After chilling three days the carcasses were air and water weighed (Brown *et al.*, 1950) and carcass measurements were made. The carcasses were then chilled for two to three days more and cut to obtain lean measurements and cut out percentages. All measurements (except loin lean area) were obtained from both halves of the carcass and the average used in the analysis. The entire carcass was cut and trimmed to obtain the percentage of primal cuts.

The following measurements and evaluations were obtained and studied:

1. Specific gravity was obtained by dividing the air weight of the carcass by the air weight minus the water weight.

2. Carcass length was measured from the anterior edge of the aitch bone to the anterior edge of the first rib.

3. The back fat measurement used for analysis was the sum of three measurements which were taken opposite the third and last ribs and the sixth lumbar vertebra. The reported mean is an average of these three measurements.

4. The loin lean area was the product of the two dimensions of the loin eye muscle exposed when the loin was bisected between the last two ribs.

5. The ham lean area was the product of the two dimensions of the lean exposed at the butt when the ham was severed from the side.

6. The percentages (based on shrunk live weight) of four primal cuts and each cut individually.

The hams were skinned about two-thirds of the way to the shank and the fat trimmed to one-fourth inch in thickness. The loins had all external fat removed except scraps at the blade and ham ends. The shoulders were trimmed New York style with all external fat removed about one-half way to the shank. Bellies were squared and trimmed as large as possible. The lower edge was trimmed to the teat line and the loin edge straightened to form a rectangle. The forward end of the belly coincided with the cut to remove the shoulder at the third rib. The posterior end was cut as long as possible after removing the ham at a line perpendicular to the long axis of the ham and half way between the aitch bone and the sixth lumbar vertebra.

The data were analyzed by groups. No attempt was made to combine the groups, since there were environmental differences between groups and minor cutting variations between seasons. It was thought that any results found from an overall analysis would be confounded due to the fact that there were breeding differences between seasons and groups in addition to the environmental differences already mentioned.

Analysis of variance was used to test mean differences. In groups one and four, mean squares for differences between litters within breeding was used in the F-test to determine the significance of differences due to breeding. In group two the mean square for individuals within line and in group three the mean square for individuals within breed and within ration were used to test the respective mean squares for breeding. In designing this experiment certain comparisons were desired. In groups three and four, where significant differences were found, these comparisons were made orthogonally.

TABLE 1. LOT MEANS FOR CARCASSES IN GROUPS ONE AND TWO

Item		Group One (Dry Lot) Duroc					Group Two (Pasture) Duroc				
		Two-line crosses					Inbred Lines				
Line		5x3	Cx3	Sx3	Tx3	Outbred	5	C	S	T	3
Number of pigs		6	6	6	6	6	4	4	4	4	5
Shrunk wt. (lbs.)		218	214	214	215	216	213	216	223	215	213
Dressing %[1]		73.4	73.4	73.2	72.6	73.4	74.0	72.0	71.9	70.7	73.4
% Primal Cuts	(x)	44.4	44.1	43.5	42.3	44.3	46.6	43.7	44.4	42.5	43.9
% Ham[2]	(x)	10.9	10.9	11.0	9.9	11.1	11.6	10.6	11.1	9.8	11.2
% Loin[2]	(x)	9.0	9.2	9.0	8.9	9.1	10.0	9.3	8.7	9.8	9.2
%Shoulder[2]	(x)	11.9	11.0	11.4	10.9	11.8	12.2	11.3	11.7	11.1	11.5
% Belly	(x)	12.6	13.0	12.1	12.6	12.3	12.8	12.4	12.8	11.7	12.1
% Fat Trim		20.6	20.2	21.0	21.0	19.9	17.8	19.7	18.4	20.4	20.3
% Lean Trim		3.4	3.8	3.4	3.6	3.8	3.8	3.7	3.8	3.1	3.6
Carcass Length, in.	(x)	29.1	28.5	29.1	29.3	28.8	29.4	27.9	29.4	29.6	28.3
Av. Backfat, in.	(x)	1.95	1.97	2.02	1.86	2.01	1.83	1.89	1.78	1.96	1.84
Specific Gravity	(x)	1.018	1.019	1.021	1.022	1.025	1.022	1.025	1.027	1.022	1.023
Loin Lean Area, sq. in.	(x)	4.06	5.19	4.22	4.22	5.80	4.85	5.44	4.58	4.84	4.92
Ham Lean Area, sq. in.	(x)	20.9	20.8	20.7	20.6	21.7	22.7	20.8	22.3	18.7	22.6

[1] Chilled carcass weight as a percentage of shrunk live weight, hogs dressed packer style with head and leaf fat removed.
[2] Very closely trimmed.
(x) Measurements analyzed statistically.

Results

Analysis of the data for ten measurements on the carcasses of the two-line crosses and outbred Durocs in group one showed no significant differences among breeding groups (table 1). However, the line crosses were not unrelated—boars from four different lines (5, C, S and T) were all bred to gilts of line 3.

In group two the T-line was significantly inferior to the average of the other four lines in percentage of ham and ham lean area (table 1). Though not significant, the Tx3 cross in group one showed the same trend. Apparently light hams typify line T Durocs. Though no other statistically significant differences were noted, there was an indication that the lines differed in certain carcass measurements. For instance, within their respective groups, line 5 carcasses showed the highest percentage of primal cuts because of heavier hams and shoulders. Line 5 also had a larger ham lean area. Line S appeared to be leaner and have heavier hams and shoulders than line C, whereas line C had a larger loin lean area and heavier loins than line S. Table 3 shows that these tendencies also appeared when boars of lines T, C, and S were crossed on 3x5 females.

The means for group three are shown in table 2. From the orthogonal comparisons the following significant differences were found:

1. Minnesota #2 cross carcasses were longer, had heavier hams, and were leaner as measured by both average back fat and specific gravity than were Duroc carcasses.

2. Outbred Durocs had heavier hams and larger loin lean area than the two-line crosses.

3. SxC and CxS line crosses had a higher specific gravity than Tx3 and 3xT carcasses.

The 53 carcasses in group four represent breeding lots that were of more diverse genetic origin than any other group studied. Table 3 gives the means for the different lots. Variation among these means for the different measurements also is considerably greater than that found in any other group. As might be expected, significant differences were found in nine out of the ten items considered. Orthogonal comparisons indicate the following differences to be significant:

1. Non-Duroc carcasses were superior to Durocs in all measurements except percentages of shoulder and belly.

2. Landrace-Poland carcasses were superior to crossbreds in all measurements except percentages of loin and belly.

TABLE 2. LOT MEANS FOR CARCASSES IN GROUP THREE

Comparison	Duroc			Crossbred
Line	SxC CxS	Tx3 3xT	OB Duroc	M2 x Inbred Duroc
Number of pigs	8	8	8	8
Shrunk weight, lbs.	222	221	220	219
Dressing %[1]	72.4	72.7	72.3	72.8
(x) % primal cuts	43.3	43.1	44.4	45.0
(x) % ham[2]	11.1	10.7	11.6	11.9
(x) % loin[2]	8.9	9.1	9.2	9.6
(x) % shoulder[2]	10.8	11.3	11.3	11.4
(x) % belly	12.5	12.0	12.4	12.1
% fat trim	20.5	21.1	19.8	18.7
% lean trim	3.4	3.4	3.2	3.4
(x) Carcass length, in.	28.7	28.9	28.8	30.2
(x) Av. B.F. thickness, in.	2.03	2.06	1.99	1.81
(x) Specific gravity	1.028	1.023	1.028	1.034
(x) Loin lean area, sq. in.	4.10	3.63	4.84	4.61
(x) Ham lean area, sq. in.	21.69	22.38	22.85	23.44

[1] Chilled carcass weight as a percentage of shrunk live weight, hogs dressed packer style with head and leaf fat removed.
[2] Very closely trimmed.
(x) Measurements analyzed statistically.

3. Outbred Duroc carcasses were inferior to line cross Duroc carcasses in six measurements, i.e., carcass length, average back fat thickness, specific gravity, and percentages of primal cuts, loin, and shoulder.

TABLE 3. LOT MEANS FOR CARCASSES IN GROUP FOUR

Comparison	Duroc						Crossbred		Landrace Poland
Line	3xT Tx3	N10x5	Cx 3–5	Sx 3–5	Tx 3–5	OB Duroc	Pol x Duroc	M2x 3–5	A
Number of pigs	8	4	6	6	6	6	6	6	5
Shrunk weight, lbs.	214	216	217	218	218	215	219	215	217
Dressing %[1]	73.3	73.1	73.7	73.4	72.7	72.8	73.7	73.5	72.7
(x) % primal cuts	45.2	43.5	44.7	44.9	43.5	43.2	46.9	45.9	49.3
(x) % ham[2]	11.5	11.0	11.1	12.1	10.7	11.6	12.6	12.4	14.2
(x) % loin[2]	9.5	8.5	9.0	8.7	8.6	8.2	9.8	9.3	10.2
(x) % shoulder[2]	11.7	11.1	11.4	11.3	11.1	10.6	11.3	11.5	12.1
(x) % belly	12.6	12.9	13.1	12.8	13.1	12.8	13.2	12.7	12.9
% fat trim	19.6	21.5	21.2	20.1	20.3	21.7	18.2	19.0	14.1
% lean trim	3.2	2.7	2.8	3.0	3.1	2.9	2.9	3.3	3.5
(x) Carcass length, in.	29.6	29.1	28.8	28.6	29.3	27.9	29.3	30.0	31.0
(x) Av. B.F. thickness, in.	1.82	2.03	2.02	1.95	1.99	2.12	1.89	1.78	1.55
(x) Specific gravity	1.029	1.024	1.025	1.026	1.023	1.020	1.032	1.028	1.038
(x) Loin lean area, sq. in.	4.32	3.52	4.24	4.24	3.83	4.22	5.23	4.78	5.78
(x) Ham lean area, sq. in.	20.88	19.58	20.62	21.44	20.05	21.28	23.17	22.58	26.64

[1] Chilled carcass weight as a percentage of shrunk live weight, hogs dressed packer style with head and leaf fat removed.
[2] Very closely trimmed.
(x) Measurements analyzed statistically.

4. Tx3 and 3xT Duroc carcasses had larger percentages of primal cuts and loin than did N10x5 Duroc carcasses.

5. Carcasses from Duroc lines S and C crossed on 3x5 had a higher percentage of ham than did Tx3–5 carcasses.

6. Sx3–5 Duroc carcasses had higher percentages of ham than Cx3–5 Duroc carcasses.

There were no significant differences between the Poland China cross and the Minnesota #2 cross carcasses. Neither were there any significant differences between carcasses from two-line cross Durocs and three-line cross Durocs.

The outbred Durocs of group four did not produce quite as good carcasses as expected. Outbreds in other group trials have been better than these and it is felt that these may not be typical of that breeding.

Discussion

The results of this study are not intended as characterizing the carcass merits of the various breeds used. It is believed, however, that the Duroc lines tested have been partially characterized with respect to their carcasses. It should be possible to predict with reasonable accuracy the relative carcass merits of certain of the lines in further crossing.

Since the numbers of carcasses were small, no attempt was made to determine standard deviations for the measurements used. It is of interest to point out, however, that the within-group variation as measured by the error mean square was very uniform for the four groups studied. Table 4 gives the error mean square for all groups. It would appear that under the conditions of this experiment the error mean squares have been well established for the characteristics studied. Apparently there is still considerable natural variation in percentage of primal cuts and ham lean area. This may be reduced by increasing the accuracy of cutting and measuring techniques.

There was never any indication of differences in percentage of belly. It was observed during the course of the study that longer or deeper carcasses had thinner bellies and short, very fat carcasses had thick bellies. Since there was no accurate measure made of quality, it is impossible to say accurately which line of breeding produced the more desirable bellies. It was noted, however, that all the carcasses of the Landrace-Poland line used in the study were too soft to be highly desirable. This line was the only one showing this carcass characteristic.

TABLE 4. MEAN SQUARES FOR BREEDING AND ERROR BY GROUPS

	Group One		Group Two		Group Three		Group Four	
Item	Breeding d.f.=4	Error d.f.=10	Breeding d.f.=4	Error d.f.=16	Breeding d.f.=3	Error d.f.=25	Breeding d.f.=8	Error d.f.=18
% Primal Cuts	4.24	2.54	9.49	3.82	6.15	3.21	19.70**	1.74
% Ham	1.47	.62	1.88*	.56	2.16**	.40	5.84**	.38
% Loin	.08	.54	1.17	.82	.66	.34	2.41**	.53
% Shoulder	1.26	.83	.61	.50	.57	.50	.95*	.36
% Belly	.74	1.78	.98	1.24	.39	.44	.29	1.09
Carcass Length	.63	.90	2.35	1.13	4.00**	.49	4.24**	.60
Σ 3 B.F. Measurement	.23	.44	.16	.48	.90**	.18	1.41**	.29
Specific Gravity	43.10°	50.73°	23.58°	35.79°	184.95°**	23.03°	151.34°**	25.84°
Loin Lean Area	3.47	1.24	.40	.40	2.34*	.31	2.50**	.44
Ham Lean Area	1.08	5.13	11.95*	3.50	4.36	4.67	23.46**	3.50

* Significant at the .05 level.
** Significant at the .01 level.
° Coded.

The evaluations used were those which more nearly measured the characteristics of interest. However, it is fully appreciated that without some measure of carcass quality, the carcass merit of any line of breeding cannot be fully characterized.

Summary

The carcasses of 136 hogs were studied to determine differences due to breeding. The study included inbred lines, two-line crosses, three-line crosses, and outbreds, all Duroc; crossbreds; and an inbred line of Landrace-Poland. Carcass measurements and evaluations included specific gravity, carcass length, back fat measurements, loin lean area, ham lean area, and percentage of four primal cuts. Differences due to breeding were found for all measurements except percentage of belly.

Landrace-Poland carcasses, though somewhat soft, were longer, leaner, and considerably higher in percentages of three lean cuts. The crossbred carcasses were, in the opinion of the workers, the most desirable carcasses in the study. The quality was good and the carcasses were longer, leaner, and higher in percentage of ham and loin than those of any other breeding except the Landrace-Poland line.

Few statistically significant differences between the breeding groups representing the Duroc breed were found. Trends, however, were noted, and it is believed that certain lines are partially characterized as a result of this study. If inbred lines being developed at the present time can be characterized as to carcass qualities, it should be possible to select lines for crossing that would yield the kind of carcasses desired.

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AGE AT PUBERTY AND RELATED PHENOMENA IN OUTBRED CHESTER WHITE AND POLAND CHINA GILTS

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PUBERTY in gilts occurs "about 5 months" of age according to Smith (1937); Rice and Andrews (1951) give a range of 3–7 months. Phillips and Zeller (1943) presented data which indicate that Poland China gilts reach puberty at approximately 6½ to 7 months. Inbred Chester White and inbred Yorkshire gilts reached puberty at an average of 8 or 9 months in recent studies at the Wisconsin Station (Warnick *et al.*, 1949; Warnick *et al.*, 1951). Squiers *et al.* (1949) reported that age at first estrus averaged 7–8 months for inbred, and 6–7 months for non-inbred gilts at the Missouri station; these were based on inbred Poland China, inbred Hampshire and outbred Durocs and their crosses.

Warnick *et al.* (1951) noted a tendency for the ovulation rate in puberal gilts to increase from the first through the third heat period; earlier, Stewart (1945) reported that gilts farrowing at about 14 months produce larger litters than gilts farrowing at one year or less than a year of age. An increase of 0.5 pigs for each 10 days increase in age at breeding of gilts was indicated by Squiers *et al.* (1950). The relative importance of the influence of ovulation rates, fertilization rates and embryonic death rates on size of litter farrowed is not known.

This study is an attempt to identify some of the causes of variation in age at puberty, ovulation rate (the number of corpora lutea formed per animal per heat period), fertilization rate (the percentage of eggs fertilized per animal per heat period), and embryonic death rate (the discrepancy, expressed as a percentage, between the estimated average number of fertilized eggs and average litter size) in two breeds of swine.

Materials and Methods

Forty-nine Chester White and thirty-seven Poland China non-inbred gilts from the 1949 spring pig crop of the University of Wisconsin swine herd were used in this study. Twenty-seven Chester Whites

and sixteen Poland Chinas were designated for slaughter early in the study and twenty-two Chester Whites and twenty-one Poland Chinas were selected for the breeding herd. This selection for the breeding herd was done by choosing the gilts which were superior in conformation and size just prior to the breeding season.

All gilts were checked for heat once daily beginning July 8. Early checking consisted of examination of vulvas for increased size and vascularity. A vasectomized boar was used for checking heat beginning on September 20. Gilts were weighed at 56 days of age and at two-week intervals from 98 days until puberty.

Those gilts which had been designated for slaughter were bred on the first day of the second estrus; with one exception, they were mated only once (one gilt was bred a second time six hours later because of an incomplete first mating). To obtain ovulation rates and fertilization rates, these gilts were slaughtered 24–48 hours after the end of second estrus. Both the first and second crops of corpora lutea could be counted at that time and most of the ova were in the oviducts or in the upper 4–6 inches of the uterine horns. A physiological salt solution was used to flush the ova from the tubes. "Flushings" were examined under a low power binocular microscope and the presence of two or more blastomeres was the criterion of fertilization. Fertilization rates could not be determined in one Chester White due to an injury at the time of mating, and in one Poland China which failed to exhibit a normal second estrus.

Breeding-herd gilts were bred on the first day of estrus during the breeding season of December 15–January 19. Most of these gilts were bred at heat periods later than the second. All gilts were mated only once during the heat period. Only one gilt did not conceive from the single breeding during the breeding season and she was rebred on January 22.

An estimate of embryonic death rate in each breed was obtained from comparisons of the average number of eggs fertilized in the slaughtered animals with the average litter sizes of those gilts retained in the breeding herd.

To determine the possible effect that selection for heavy body weight would have on earliness of sexual maturity the following correlations with age at puberty were calculated:

(1) Simple correlations with weight at 98 days, and at two-week intervals thereafter,

(2) Simple correlations with gains in each of the two-week periods, and

TABLE 1. AGE AND WEIGHT OF GILTS AT FIRST HEAT

Breed	No.	Age			Weight		
		Average (days)	Standard Deviation	Coeff. of Variation	Average (lbs.)	Standard Deviation	Coeff. of Variation
Poland China	37	201	17.2	8.6	224	31.3	14.0
Chester White	49	204	16.0	7.9	212	24.2	11.4

(3) Multiple correlations with body weight at each of the above times and gain during the subsequent two-week period.

Results and Discussion

Age and Weight of Gilts at Puberty

The Poland China gilts averaged within three days of the same age at first estrus as the Chester Whites ($P>0.05$). There was, however, a significant difference in weights at first heat, the Poland Chinas averaging twelve pounds heavier than the Chester Whites (table 1). Within each breed, age at first heat is more constant than weight at first heat; this is shown by the significantly smaller coefficients of variation for age than for weight at first heat.

Length of First Estrual Cycle in Gilts

The average length of first estrual cycle in the Chester White gilts was 20.5 days as compared to 21.0 days in the Poland China gilts (table 2). The small difference between the means was not significant.

TABLE 2. LENGTH OF FIRST ESTRUAL CYCLE IN GILTS.

Length of cycle (days)	Frequency	
	Chester White	Poland China
18	2	0
19	8	0
20	19	11
21	9	14
22	6	8
23	2	2
24	0	0
25	2	0
Total No.	48	35
Mean (days)	20.5	21.0
Standard deviation	1.48	0.95

$X^2=7.33$ $P\leqq 0.05$

The length of individual estrual cycles in the Chester White gilts ranged from 18 to 25 days, inclusive, with a standard deviation of 1.48 days; the range in the Poland China gilts was from 20 to 23 days and the standard deviation 0.94 days. This is a highly significant difference in variability.

Ovulation Rates

The difference in ovulation rates between the breeds was highly significant at both first and second heat periods; the numbers of

TABLE 3. OVULATION RATES OF GILTS AT FIRST AND SECOND HEAT PERIODS

Comparison	Chester White		Poland China	
	First Ovulation	Second Ovulation	First Ovulation	Second Ovulation
No. Animals	27	27	16	16
Avg. No. Corpora Lutea	12.3	13.9	9.8	11.0
Standard Deviation	2.42	2.79	2.37	1.15
Correlation Between First and Second Ovulation	.70**		.54*	
Pooled Correlation	.65**			

* P≦0.05.
** P≦0.01.

corpora lutea were greater in the Chester White by 2.5 and 2.9, respectively (table 3). Likewise, the increased ovulation rate at second heat over the first was highly significant within each breed. The average increase was 1.6 eggs in the Chester White and 1.2 eggs in the Poland China. The increase in rate from first to second ovulation did not differ significantly between breeds.

The correlations between first and second ovulation of 0.70 for the Chester White and of 0.54 for the Poland China were significant (table 3) and these correlations did not differ significantly from each other. When these estimates of repeatability of ovulation rate were pooled it was found that the average correlation, 0.65, differed ($P<.01$) from the estimate (0.15—Lush and Molln, 1942) of repeatability of number of pigs farrowed. There is likely to be less opportunity for environmental effects on ovulation rate than on litter size because

two successive ovulations are separated by a shorter and more uniform length of time than are successive litters.

Neither age nor weight at either first or second heat period seemed to be very closely associated with ovulation rate as shown by the low correlation coefficients, only one of which was significant (table 4). It is suspected therefore that calendar age is not as important in determining ovulation rate as is "sexual age"—indicated above by the change in number of ovulations from first to second heat.

TABLE 4. ASSOCIATION OF AGE AND WEIGHT WITH OVULATION RATES IN GILTS

Comparison	Chester White		Poland China	
	First Ovulation	Second Ovulation	First Ovulation	Second Ovulation
Correlation Between Ovulation Rate and Age	—.20	—.05	—.09	—.34
Correlation Between Ovulation Rate and Weight	.09	.30	—.28	—.52*

* $P \leq 0.05$.

Squiers, *et al.*, 1950, reported an increase of .5 pig for each 10 days increase in age at breeding, due in part to an association between ovulation rate and age. Their data were obtained from gilts which averaged 225 days of age, suggesting that at least some of their animals were slaughtered following the second or third heat. If such were the case, then the increased ovulation rate which they noted with increased age at breeding might have been due to effects of factors associated with sexual age, i.e., age following puberty, rather than to the influence of chronological age.

It was observed that the left ovary had a higher ovulation rate than the right, as reported by Warwick (1926). The left ovary accounted for 58.7% and 55.0% of 310 and 133 ovulations in Chester White and Poland China gilts, respectively.

Fertilization Rates

Fertilization rates at second heat were obtained on all of the slaughtered gilts, except the two noted under material and methods. Fertilization could not be expected in two other animals, one of each breed, which showed bilateral salpingitis and bursitis at slaughter;

TABLE 5. FERTILIZATION RATES

Breed	No. Animals	Range of Fertility in Individual Gilts (%)	Mean Fertility for Entire Group (%)
Chester White	25	85.7–100	98.1
Poland China	14	67.0–100	92.6

these two gilts also were excluded. In 21 of the 25 Chester White gilts, all recovered eggs were fertilized, while the lowest fertility percentage for any of these gilts was 85.7 (table 5). Similarly, in the Poland Chinas, 8 out of 14 gilts showed complete fertility with the lowest percentage being 67. Only two boars were used in each breed so it is questionable whether this constitutes a satisfactory breed comparison. The percent of eggs recovered was 82.4 for the Chester White and 85.3 for Poland China gilts.

Conception Rates and Embryonic Death Rates

Only one of the 43 animals selected for the breeding herd required a second service for conception, giving 97.7% conception on first service. Average size of litter farrowed was 10.27 for the Chester White and 6.34 for the Poland China gilts (table 6). Estimates of embryonic death were obtained by comparing these litter sizes with the average numbers of eggs fertilized in the slaughtered groups. The percent of embryonic death was found to be 24.9 for the Chester White and 37.8 for the Poland China gilts. The difference in presumed embryonic death rates of the two breeds was significant ($P<0.05$).

These estimates of embryonic death may be low for the reason that many of these gilts were bred at the third and fourth heat periods as compared with the slaughtered gilts which were bred at second heat, at which time ovulation rates are probably lower than at later heat periods. The error should be greater for the Chester Whites, however, since nearly 24% of the Poland Chinas were actually

TABLE 6. CONCEPTION RATES AND EMBRYONIC DEATH RATES IN BREEDING-HERD GILTS

Breed	No. Animals	No. Conceiving First Service	Maximum Litter Size Possible[1]	Average Litter Size	% Embryonic Death
Chester White	22	22	13.67	10.27	24.9
Poland China	21	20	10.19	6.34	37.8

[1] Based on ovulation rates and fertilization rates at second heat period in the slaughtered animals.

bred at second heat, while none of the Chester Whites were bred at second heat. It is possible that these estimates of embryonic death if measured with equal accuracy in both breeds would not be significantly different.

Effect of Sexual-age of Gilts at Conception on Litter Size

The limited data available (table 7), suggest that litter size increases with the order number of the heat period at which gilts

TABLE 7. SIZE OF LITTERS FARROWED BY GILTS BRED AT DIFFERENT HEAT PERIODS

Breed	Variable	Heat Period of Conception			
		1	2	3	4
Chester White	No. Gilts Farrowing	1	—	9	11
	Avg. Litter Size	9.0	—	10.3	10.36
Poland China	No. Gilts Farrowing	1	5	5	9
	Avg. Litter Size	6.0	5.4	7.2	7.11

conceive, up to the third period when it tends to level off. A similar tendency was observed in inbred swine at this station by Warnick *et al.*, 1951.

Effect of Season of Farrow on Age at Puberty

Gilts which are born later in the farrowing season tended to reach puberty at a younger age, as shown by the highly significant negative correlations between date of farrow and age of puberty, —0.37 and —0.49 for the Chester Whites and Poland Chinas, respectively (b=—.49 and —0.61). This is in accord with previous work reported by Wiggins *et al.* (1950). A similar seasonal influence on age at puberty in rabbits (Hammond, 1925), chickens (Upp and Thompson, 1927) and in pigeons (Riddle, 1931) has been reported.

Association of Growth Rates with Age at Puberty

If puberty appears earlier in faster growing gilts, one should be able to predict, within limits, age at puberty from body weight at a given age. Correlations between body weight at different ages and

TABLE 8. CORRELATION COEFFICIENTS OF WEIGHT AT DIFFERENT AGES AND AGE AT PUBERTY IN GILTS

Breed	No. Animals	Ages of Gilts (days) 98	112	126	140	154	168	182	196
Chester White	49	—.10	—.15	—.21	—.38**	—.34*	—.39**	—.40**	—.38**
Poland China	37	—.09	—.12	—.23	—.17	—.18	—.18	—.24	—.29

* $P \leq 0.05$.
** $P \leq 0.01$.

age at puberty (table 8) for the Poland China were all negative, but none was significant. In the Chester Whites, also, all the correlations were negative. They were, however, significant at each age from 140 days to 196 days but were not significantly heterogeneous. This does not allow us to conclude that there was any better predictive value from weight at one age than at another. There was no significant difference between breeds in the correlations of body weight (at any age) and age at puberty.

Correlations between gains over different 14 day periods and age at puberty (table 9) are insignificant except during the 126–139 day period for the Chester White and the 112–125 day period for the Poland China. These correlations were not significantly heterogeneous. Likewise, there was no evidence of breed differences in these correlations.

Multiple correlations of weight at a given age and total gain during the subsequent 14-day period with age at puberty (table 10) did not indicate that a combination of these two factors was any more closely associated with age at puberty at one age than at any other age. Within breeds, the group of multiple correlations was not heterogeneous, nor was there any significant difference between the correlations in the two breeds, at any age.

TABLE 9. CORRELATION COEFFICIENTS OF GAINS IN WEIGHT DURING 14-DAY INTERVALS WITH AGE AT PUBERTY

Breed	No. Animals	14-Day Intervals 98–111	112–125	126–139	140–153	154–167	168–181	182–195
Chester White	49	—.19	—.26	—.38**	+.05	—.10	—.13	—.14
Poland Chinas	37	—.16	—.46**	+.14	—.10	—.05	—.25	—.30

** $P \leq 0.01$.

TABLE 10. MULTIPLE CORRELATION COEFFICIENTS OF WEIGHT AT DIFFERENT AGES AND GAIN DURING SUBSEQUENT 14 DAY PERIOD WITH AGE AT PUBERTY

Breed	No. Animals	Ages of Gilts (days) 98	112	126	140	154	168	182
Chester White	49	.20	.27	.43**	.38*	.34	.43**	.44**
Poland China	37	.16	.47*	.34	.19	.18	.28	.36

* $P \leqq 0.05$.
** $P \leqq 0.01$.

Ages and Weights at Puberty of Gilts by Different Sires

There were no significant differences in ages at puberty between gilts by different sires; since the average age at puberty was essentially the same for the two breeds, the sums of squares were pooled (table 11).

Differences in weight at puberty between gilts by different sires were significant in the Chester Whites but not in the Poland Chinas (table 12).

No estimates of heritability are reported from the analyses summarized in tables 11 and 12 because of the wide fiducial limits which would be associated with estimates based on such small numbers.

Summary

Data on age at puberty are presented on 49 Chester White and 37 Poland China gilts. There was not a significant difference in age at puberty between these two breeds but the Poland Chinas were significantly heavier at puberty than the Chester Whites.

TABLE 11. ANALYSIS OF VARIANCE IN AGE AT PUBERTY BY BREEDS, SIRES AND LITTERS

Source of Variation	d.f.	Mean Squares
Breeds	1	279
Sires within breed	3	335
Litters within sire	18	533
Pigs within litter	57	213

TABLE 12. ANALYSIS OF VARIANCE OF WEIGHT AT PUBERTY BY SIRE AND LITTERS

Source	Chester White d.f.	m.s.	Poland China d.f.	m.s.
Sires	1	4098*	2	2487
Litters Within Sires	11	342	7	1166
Pigs Within Litters	33	593	24	898

* $P \leqq 0.05$.

Length of the first estrual cycle was more variable in Chester Whites than in Poland Chinas.

Chester White gilts ovulated a significantly greater number of eggs at first and second heat periods than did the Poland Chinas. Within each breed the increase of second ovulation over the first was highly significant. Neither age nor weight at first or second heat periods was closely associated with ovulation rate. In each of these breeds, the left ovary ovulated more eggs than did the right ovary.

Fertilization rate was higher and embryonic death rate was lower in Chester Whites than in Poland Chinas. Average size of litter farrowed was 10.27 for Chester White and 6.48 for Poland China.

Gilts born later in the farrowing season tended to reach puberty at a younger age.

It was not possible to predict age at puberty in these animals from a knowledge of body weight with any greater accuracy at one age than at any other age.

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EFFECT OF APF CONCENTRATE CONTAINING AUREOMYCIN ON GESTATING, LACTATING, AND GROWING SWINE[1, 2]

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THE stimulatory effect of aureomycin on the growth of swine was first observed by Jukes *et al.* (1950) and Carpenter (1950), who observed also that the antibiotic was effective in controlling a diarrhea that commonly occurs in young pigs. It is postulated that the antibiotic in some manner favorably influences the intestinal flora of the pig, possibly by inhibiting undesirable bacteria that have a retarding effect on the growth rate. Because many pigs die early in life from various causes, some of which may be related to the health of the sow during the gestation period, it seemed possible that feeding an antibiotic during the gestation period might improve the well-being of the sow and increase the size and livability of the pigs at birth.

The experiments reported herein were conducted to study (1) the effect on the nursing pig of feeding an APF concentrate containing aureomycin to sows during gestation and lactation, and (2) the effect of feeding different dietary levels of aureomycin as a crude concentrate to weaned pigs.

Experimental Procedure and Results

The diets used during the gestation and lactation periods were as follows:

	Diet I	Diet II	Diet III
Ground yellow corn	35	30	44
Ground oats	21	21	8
Ground barley	21	21	—
Dried skim milk	—	—	2
Wheat bran	—	—	6
Alfalfa leaf meal	10	10	20
Tankage	4	6	8
Linseed	3	5	4
Soybean oil meal	4	5	6
Bone meal	1	1	1
Salt (iodized)	1	1	1
Cod liver oil	—	—	0.25

[1] This work was supported in part by Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York, who also furnished the APF supplement and the pure aureomycin hydrochloride.

[2] Hormel Institute publication No. 61.

[3] The Hormel Institute, Austin, Minn.

All the sows were fed diet I during the first 85 days of the gestation period and diet II during the last 30 days. Each sow was allowed seven pounds of feed daily during gestation, equal portions being given morning and night. Diet III was self-fed during the lactation period.

The source of aureomycin was Lederle APF feeding supplement no. 5. Different batches of supplement were used during the course of the experiments, but all contained about 1.3 mg of vitamin B_{12} and 1.9 gm of aureomycin per pound.

All animals were from the Hormel Foundation herd. Sows and gilts were bred to unrelated boars and were put in dry lots about 50x200 ft. in size, each provided with a 12x16 ft. frame hog house for sleeping quarters. Fresh water was available at all times. The dams farrowed in a heated hog house in 6x8 ft. pens with concrete floors, maintained at 70° F. Ten to 14 days after parturition, the sow and litter were moved to pens 6x15 ft., with a 3 ft. creep, where they remained until the pigs were 56 days old.

Experiment I. Effect of feeding APF concentrate containing aureomycin to sows during gestation and lactation.

Breeding in the Hormel Foundation herd is conducted on a year-around schedule, averaging one sow per day. On March 1, the animals bred to farrow in April and May were divided into four lots. Lots 1 and 3 contained 14 and 13 animals respectively that served as controls, and lots 2 and 4 contained 14 and 12 animals respectively that served as test animals (table 1). All animals had been on the same dietary regime up to March 1. During the gestation period the control dams in lots 1 and 3 were fed diets I and II, as described above; the test dams in lots 2 and 4 were fed the same diets plus 0.5% APF supplement. Because the breeding dates were staggered, the diets containing the antibiotic supplement were fed to lots 2 and 4 over a period of 49 to 90 days prior to parturition. After parturition the control dams were fed diet III and the test dams received the same diet containing 0.5% APF concentrate. The pigs nursing the control dams were fed a creep diet of rolled oats while those nursing the test dams were fed the same creep diet plus 1% APF concentrate.

A summary of the litter performance of the dams is given in table 1. Although the control sows that farrowed in April (lot 1) had an average of 2 pigs per litter more than did the test sows (lot 2), the number of pigs farrowed per sow during May was essentially the same for control and test lots (3 and 4). The number of dead pigs farrowed per litter was the same for all four lots. Since the sows that farrowed in May were

fed the APF supplement over a longer period than those that farrowed in April, the results indicate that the antibiotic in the supplement does not affect litter size nor the number of pigs born dead. The average birth weights of the pigs farrowed by the dams in all four lots were the same.

The average weaning weight of the pigs in lots 2 and 4 was 36 lbs. compared to 25 lbs. for the pigs nursing the control dams (lots 1 and 3). The incidence of diarrhea was much greater for the pigs nursing the control dams than for those nursing the test dams, especially during

TABLE 1. EFFECT OF FEEDING AN APF CONCENTRATE CONTAINING AUREOMYCIN TO SOWS DURING GESTATION AND LACTATION

	April Farrow		May Farrow	
	Control lot 1	Test lot 2[1]	Control lot 3	Test lot 4[1]
Number of sows	14	14	13	12
Average weight loss during lactation	88 lb	61	65	66
Pigs born alive per litter	10.4	8.4	9.0	9.3
Pigs born dead per litter	0.5	0.5	0.5	0.5
Average weights: at birth	2.7 lb	2.8	3.0	3.1
at 56 days	24.7 lb	34.6	25.2	37.5
Number weaned per litter	6.4	6.5	7.0	6.4

[1] Diet contained 0.5% and creep diet 1% APF supplement.

the latter part of the 56-day period. The control dams in the April farrow lost an average of 27 lbs. more than the test dams during the lactation period but the weight loss for the two groups that farrowed in May was the same. The control dams consumed an average of 717 and 644 lbs. of feed during the lactation period for the April and May farrows respectively, compared to an average of 760 and 671 lbs. for the test dams during the same periods. The pigs nursing the control dams consumed an average of 106 lbs. of creep feed compared to 190 lbs. for the pigs nursing the test dams.

Experiment II. Effect of feeding an APF supplement during lactation

Experiment I showed that pigs nursing dams fed an APF supplement containing an antibiotic gain faster than pigs nursing control dams. Because pigs eat a relatively small amount of feed during the nursing period, especially during the first four weeks, it seemed possible that the antibiotic in the sow feed was transferred to the pig through the milk in sufficient quantities to stimulate growth. To test this hypothesis, three groups of sows and litters were placed on the dietary regimes indicated in table 2. The basal diet for the sows was diet III,

and for the nursing pigs, rolled oats. All sows were put on the test diets immediately after parturition, and the creep diet was offered to the pigs 10 to 14 days after birth. The data, summarized in table 2, show that pigs nursing dams fed a diet containing 0.5% APF do not grow any faster than pigs nursing control dams, indicating that the antibiotic does not get into the milk in sufficient amounts to cause growth stimulation. A comparison of the weaning weights suggests that all the stimulation was due to the presence of APF in the creep diet.

TABLE 2. THE EFFECT OF FEEDING AN APF CONCENTRATE CONTAINING AUREOMYCIN TO SOWS AND PIGS DURING THE 56-DAY LACTATION PERIOD

Diet supplement		Number of	Average number pigs per litter		Average weaning	Average pounds of feed consumed	
Dams	Pigs	litters	Farrowed	Weaned	weight	Dam	Pigs
					lbs.		
None	None	9	9.4	7.0	29.0	629	145
0.5% APF	None	7	9.9	6.9	28.6	651	146
0.5% APF	1% APF	9	8.8	7.1	35.2	641	223

Experiment III. The effect of feeding aureomycin hydrochloride to nursing and weaned pigs

The above results demonstrate that feeding the APF concentrate to nursing pigs in a creep diet increases the weaning weight of pigs. Because the APF concentrate contained vitamin factors as well as aureomycin, a test was conducted to determine whether feeding pure aureomycin in a creep diet would stimulate the growth of suckling pigs. For this test, four groups of litters were fed a creep diet plus 0, 2, 4, and 8 grams of aureomycin hydrochloride per 100 lbs. of feed, beginning on the 28th day of the nursing period. Table 3 shows that pure aureomycin stimulated the growth of nursing pigs. Although the number of litters per group was small, it seems apparent that 2 gm of aureomycin per 100 lbs. of feed is as effective as 4 or 8 grams. Some of the pigs receiving aureomycin developed diarrhea, but the diarrhea was much more marked in pigs fed the control diet.

The effect of different levels of aureomycin in the form of an APF concentrate on the growth of weaned pigs that had been fed the antibiotic during the nursing period was studied by using the pigs weaned from the sows in the gestation-lactation experiments. The basal diet fed during the growing period consisted of ground yellow corn 41%,

ground oats 20%, alfalfa meal (dehydrated) 10%, soybean oil meal 7%, tankage (60%) 10%, wheat middlings 10%, commercial mineral 2%, and irradiated yeast (9000 USP units of vitamin D per gram), 0.0125%.

At weaning time the pigs farrowed during April were divided into four lots with the pigs distributed as evenly as possible with respect to size, sex, and litter. They were housed in a metal hog barn in 8x25 ft. pens provided with 25x25 ft. concrete runways. The pigs in lots 1 and 2 (table 4) were weaned from the control dams (lot 1, table 1), and those in lots 3 and 4 were weaned from the test dams (lot 2, table 1) and had received 1% APF supplement in the creep diet. The pigs in lots 1 and 3 were fed the basal diet, and those in lots 2 and 4 the basal diet plus 1% APF supplement. Because the pigs were weaned

TABLE 3. EFFECT OF FEEDING AUREOMYCIN HYDROCHLORIDE TO PIGS DURING NURSING PERIOD

Diet supplement per 100 lbs. of rolled oats[1]	Number of litters	Average pig weight		Average gain
		28 days	56 days	
		lbs.	lbs.	lbs.
None	4	12.1	21.9	9.8
2 gm aureomycin HCl	5	11.7	33.1	22.4
4 gm aureomycin HCl	4	12.8	31.3	18.5
8 gm aureomycin HCl	5	13.5	31.3	17.8

[1] Supplement started on 28th day.

on different days, not all the pigs were on the test for the same number of days; average days on test was 21.

Table 4 shows the growth-promoting effect of the APF concentrate on weaned pigs. The pigs that received APF concentrate after weaning made faster gains than litter mates fed the basal diet, regardless of the diet fed during the nursing period. When the APF supplement was removed from the diet at the time of weaning, the efficiency of feed utilization decreased from 2.82 to 4.59 lbs. feed per lb. gain. The latter figure is essentially the same as that observed for weaned pigs that had not previously received any APF concentrate (lot 1). The incidence of diarrhea was high for pigs in lot 1, but was practically nil for pigs in lot 2 at the end of the test period. The pigs in lot 4 never developed diarrhea, but signs of diarrhea were evident for pigs in lot 3 by the end of the 3-week test period.

At the end of the test period, the pigs in lots 1, 2, 3 and 4 (table 4) were divided into 16 lots, and fed the diets shown in table 5. The results show that the growth rate of pigs that had never been fed an

TABLE 4. EFFECT OF FEEDING AN APF SUPPLEMENT CONTAINING AUREOMYCIN ON GROWTH OF PIGS[1]

Lot no.	Creep supplement	Diet after weaning	Number of pigs		Average weight		Average daily gain, lbs.	Feed per lb. gain
			Start	Finish	Start lbs.	Finish lbs.		
								lbs.
1	None	Basal	45	43	24.2	33.0	0.33	4.87
2	None	Basal+1% APF	45	45	25.6	38.3	0.55	3.01
3	1% APF	Basal	45	43	34.8	46.9	0.47	4.59
4	1% APF	Basal+1% APF	45	44	35.5	54.0	0.74	2.82

[1] Test period about 3 weeks post-weaning.

APF concentrate can be stimulated by feeding a diet containing 0.25% APF concentrate, but maximum stimulation occurs with 0.5% (lots 5, 6, 7 and 8). When the dietary level of APF was reduced from 1% to 0.5% three weeks after weaning, there was no decrease in the rate of

TABLE 5. EFFECT OF FEEDING DIFFERENT LEVELS OF APF SUPPLEMENT CONTAINING AUREOMYCIN ON THE GROWTH OF PIGS

Lot no.	Diet supplement	Number of pigs	Average weight at start	Average daily gain (4 weeks)	Feed/lb. of gain
			lbs.	lbs.	lbs.
	Pigs that had received the basal diet only prior to test (from lot 1)				
5	None	8	38.0	0.69	4.85
6	0.25% APF	7	36.5	1.01	3.98
7	0.50% APF	8	36.0	1.13	3.45
8	1.00% APF	8	35.4	1.14	3.33
	Pigs that had received 1% APF supplement for only 3 weeks prior to test (from lot 2)				
9	None	10	42.6	0.78	4.98
10	0.25%	10	39.0	0.92	4.33
11	0.50% APF	10	41.1	1.09	3.63
12	1.00% APF	10	40.9	1.11	3.68
	Pigs that had received basal diet for only 3 weeks prior to test (from lot 3)				
13	None	10	47.9	0.73	5.55
14	0.25% APF	10	49.2	1.06	4.07
15	0.50% APF	10	49.4	1.01	4.34
16	1.00% APF	10	48.9	1.09	4.04
	Pigs that had received diets containing 1% APF supplement prior to test (from lot 4)				
17	None	10	56.8	0.76	5.52
18	0.25% APF	10	56.8	0.94	4.80
19	0.50% APF	10	56.3	0.94	4.55
20	1.00% APF	10	56.1	1.08	4.22

gain or in feed utilization, but reducing the level to 0.25% or eliminating the concentrate entirely reduced the rate of gain and decreased feed efficiency. The incidence of diarrhea increased when the level of APF concentrate was decreased.

The rate of gain and efficiency of feed utilization of pigs that had been fed a diet containing APF during the nursing period and a diet with no APF for three weeks after weaning was increased by again including 0.25% of the APF concentrate. Higher levels gave no additional stimulation. Reducing the dietary level of APF concentrate to less than 1% after a three-week post-weaning period reduced the rate of gain and decreased the efficiency of feed utilization (lots 17, 18, 19 and 20).

TABLE 6. EFFECT OF FEEDING DIFFERENT LEVELS OF AN APF SUPPLEMENT CONTAINING AUREOMYCIN TO WEANED PIGS FED A CREEP DIET CONTAINING 1% OF THE APF SUPPLEMENT DURING THE NURSING PERIOD

Lot no.	Diet supplement	Number of pigs		Average weight		Average daily gain (9 weeks)	Feed/lb. of gain
		Start	Finish	Start	Finish		
				lbs.	lbs.	lbs.	lbs.
21	None	20	19	34.1	68.0	0.57	4.72
22	0.25% APF	19	19	37.9	69.7	0.62	4.92
23	0.50% APF	19	19	37.7	77.8	0.78	4.38
24	1.00% APF	20	20	40.1	98.6	1.05	3.46

The effect of feeding different levels of the APF supplement to weaned pigs immediately after weaning was studied by using the pigs that farrowed during May (lot 4) and had been fed a creep diet containing 1% APF. The pigs were distributed among lots 21, 22, 23 and 24 as equally as possible according to weight, sex, and litter. They were housed in 12x16 ft. frame houses with 25x25 ft. attached concrete runways. Fresh water and feed were available at all times. The feeding regime indicated in table 6 was continued for nine weeks. In general, as the level of APF concentrate increased, the rate of growth and efficiency of utilization of feed increased. Most of the pigs in lots 21 and 22 developed diarrhea after about three weeks, whereas the pigs in lots 23 and 24 did not exhibit diarrhea during the test period.

Summary

The 56-day weaning weight of pigs was increased from 25 to 36 pounds by feeding sows a diet containing 0.5% of an APF supplement

containing aureomycin and providing a creep diet that contained 1% of the supplement. The growth stimulation was not due to any substance transferred to the pigs through the milk, but could be attributed entirely to the aureomycin present in the APF supplement included in the creep diet, as was demonstrated when a similar growth stimulation was obtained by feeding a creep diet containing pure aureomycin.

Feeding an APF supplement containing aureomycin for 49 to 90 days prior to parturition did not affect litter size or size and livability of newly born pigs.

Under the environmental conditions imposed, the growth rate and efficiency of feed utilization of weaned pigs fed a creep diet containing aureomycin during the nursing period can be stimulated by feeding diets containing aureomycin. Reducing the level of antibiotic supplement at weaning time or three weeks after weaning resulted in a decrease in rate of gain and efficiency of feed utilization and an increase in the incidence of diarrhea.

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THE EFFECTS OF PROGESTERONE UPON OVARIAN FUNCTION IN GILTS[1]

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NUMEROUS workers have shown that one action of progesterone is the prevention of estrus. It has seemed worth exploring whether this action may be utilized in control of the estrual cycle in farm animals.

The administration of 10 mg. of progesterone daily to the ewe inhibited heat and ovulation and all animals came into estrus 3 to 4 days after injections ceased (Dutt and Casida, 1948). Progesterone can be used to synchronize the estrual periods in ewes so that all animals of a group may be bred at nearly the same time (O'Mary *et al.*, 1950). Christian and Casida (1948) and Willett (1950) found that heat can be inhibited in dairy heifers by injecting 50–100 mg. of progesterone daily and all animals so treated came into heat 4 to 7 days later.

Varying the dosage of hormone in both sheep and cattle has given some suggestion of abberant ovarian function. Dutt and Casida (1948) found some follicles of excessive size in ewes injected with 5 mg. hormone daily. Further, when the dosage of progesterone in heifers is reduced to 12.5 or 25 mg. per day, follicular growth has occurred without heat or ovulation during the treatment period (Ulberg, Christian and Casida, in press).

Thus it would appear that a characterization is needed of the responses of a given species at various dosage levels. This report deals with a study made to determine the effects of four dosages of progesterone when injected into gilts.

Material and Methods

Dosages of 12.5, 25, 50 and 100 mg. daily of progesterone have been studied using a total of 43 gilts. Most of these animals were obtained from a local livestock market over a period of about nine months. They usually weighed 175 to 250 pounds and several breeds from many different farms were represented.

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These animals were brought to the University barns where heat checks were made twice daily with a vasectomized boar; no treatment was given the animals until they had shown two estrual periods. Only those gilts were used which had intervals of 18 to 22 days between the two periods; this was an attempt to eliminate animals with an abnormal endocrine system.

The first day that an animal was in heat was considered as day-1 for indicating stages of the estrual cycle. The injections of progesterone (PAC)[2] were started on day-15 or -19 and unless otherwise noted were continued through day-28. The hormone-bearing material was diluted in corn oil to make a concentration of activity equivalent to 25 mg. of progesterone in each cc. of solution. Injections were made daily with a 2 inch, 18 gauge hypodermic needle into the back fat just behind the shoulder of the animal.

The majority of the animals were slaughtered 24–48 hours after the end of the post-treatment estrus. (An animal that came in heat during treatment was slaughtered 24–48 hours after the end of that estrus.) If no heat was shown, the animal was slaughtered seven days after the end of treatment. A few of the individuals were varied from this schedule to furnish further information on what was happening in the ovaries. When a gilt was slaughtered her ovaries were examined for the number and the size of follicles and corpora lutea that they contained. An observational laparotomy was made on each of four animals some time prior to slaughter.

Results and Discussion

Thirty of the forty-three gilts were divided into six groups of five animals each (table 1). One group was placed on each dosage (12.5, 25.0, 50.0 and 100.0 mg. daily) starting on day-15 of the estrual cycle and one group each on dosages of 12.5 and 50.0 mg. daily starting on day-19 of the cycle.

Injections started on day-15

The 12.5 mg. dosage appeared to have little if any effect on inhibition of either heat or ovulation (figure 1). There was one exception; one gilt showed no heat until day-33, and when slaughtered two days later, cystic follicles were found. She had failed to ovulate. Her record increased the average estrual cycle length for the group markedly (table 1).

[2] "Progestationally Active Concentrate" furnished by The Glidden Company, Chicago, Illinois.

The gilts receiving the daily dosage of 25 mg. gave the greatest variation in response (figure 2 and 3). Heat was inhibited in all cases for at least 3–5 days after the end of treatment, the average, for the animals coming into heat being 4 days after the last injection. One animal in this group failed to show signs of heat up to seven days post-treatment; her ovaries on slaughter contained 4 corpora lutea which appeared to be more than 3 days old, indicating that she may have had a "quiet" ovulation during the treatment period. At the time of slaughter the remaining four gilts contained, on the average, 16.0

TABLE 1. THE EFFECT OF PROGESTERONE FOR 14 DAYS UPON ESTRUS, FOLLICULAR GROWTH AND OVULATION

Daily Dosage	Day of cycle-beginning treatment	Estrus by day-35		Follicles over 20 mm.		Corpora Lutea	
		No.[1]	Avg. day of cycle[2]	No.[1]	Avg. no./gilt[2]	No.[1]	Avg. no./gilt[2]
mg.							
12.5	15	5	23.2[3]	1	1.0	4	15.5
25.0	15	4	32.0	3	7.7	4	16.0
50.0	15	1	34.0	4	12.2	2	8.0
100.0	15	5	34.4	0	—	5	15.6
12.5	19	5	20.4	0	—	5	13.8
50.0	19	3	20.0	2	10.0	2	14.5

[1] The figure indicates the number of gilts out of five which showed the characteristic.
[2] The average is based upon the number of gilts showing the characteristic.
[3] Only one gilt failed to come into heat during the treatment interval and her estrus was on day-33.

corpora lutea per animal. Eggs were recovered from the oviducts indicating that these corpora were from recent ovulations. However, ovulation in animals on this dosage seemed to be "incomplete" (figure 3). Three animals had ovaries which contained one or more follicles over 20 mm. in diameter at the time of slaughter (table 1); only one gilt out of the five had ovaries with no follicles over 8 mm. in diameter.

Heat was suppressed in all gilts during the treatment period when 50.0 mg. of the hormone were injected daily. Only one gilt out of five (20%) showed estrus within seven days after treatment. Her reactions were similar to those of gilts on the 100.0 mg. dosage discussed below. The other four gilts did not come into heat and were therefore slaughtered seven days after the end of treatment. Their ovaries contained on the average 12.2 follicles over 20 mm. in diameter (table 1; figures 4 and 5). Some of these follicles reached a diameter of 50 mm. The follicular walls, in many cases, had become highly vascularized

and in many instances the liquor folliculi had become infiltered with red blood cells.

The gilts receiving a daily injection of 100 mg. gave the most consistent response. Heat and ovulation were prevented during the treat-

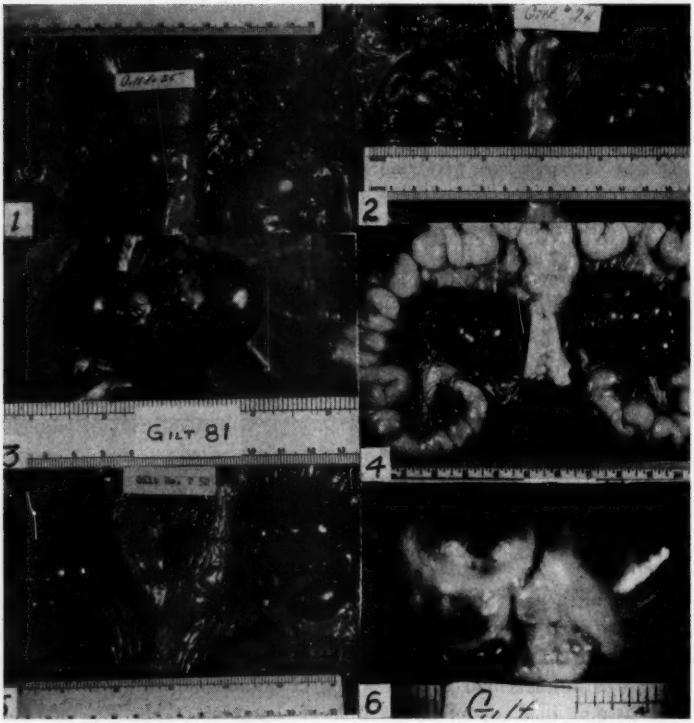

Figure 1. Daily injections of 12.5 mg. No apparent effect. Ovulation during treatment.

Figure 2. Daily injections of 25.0 mg. Very nearly complete post-treatment ovulation.

Figure 3. Same treatment as figure 2. A close-up of one ovary, showing partial post-treatment ovulation plus some cysts.

Figure 4. Daily injections of 50.0 mg. Seven days post-treatment, no CL present, many large cysts.

Figure 5. Same treatment as figure 4. Shows more variation in the size of the follicles.

Figure 6. Same treatment as figure 4. Close-up of one ovary 26 days post-treatment. Still no CL present, the structures present are very variable.

ment interval. All five animals of the group came into normal-appearing heat on the sixth or the seventh day after injections were stopped, the average being 6.4 days (table 1). At the time of slaughter all gilts had ovulated "completely," with only a few small follicles (less than 3 mm. in diameter) remainng in their ovaries. No attempt was made to mate these animals at the time of heat, therefore no information was obtained on fertility.

Injections started on day-19

The 12.5 mg. dosage had no apparent effect on the five gilts used in this group. The length of the estrual cycle and the occurrence of heat and ovulation were all judged to be within the range found in untreated gilts.

The dosage of 50 mg. had no inhibitory effect on estrus in three gilts, all coming into heat on the day following the first injection. Two of them ovulated normally and the third had not yet ovulated when slaughtered 24–48 hours after the end of estrus; her ovaries contained 23 follicles in the 6–10 mm. size range. The two remaining gilts of the five reacted the same as if treatment had begun on day-15 of the cycle, i.e., they showed no estrus and their ovaries contained many follicles over 20 mm. in diameter with no corpora lutea. The response appeared to be all-or-none. It suggests that in order for the treatment to be effective it must be started at least twenty-four hours before the animal is to come into heat. It is also possible that there is a critical stage in proestrus at which administration of progesterone will hasten estrus. Everett (1948) has shown this to be the case in the rat. Pfeiffer (1950) suggests that 0.5 mg. progesterone daily, for 3–6 days, will cause ovulation in an anovulatory monkey.

A possible explanation for the differences observed in the 15 day gilts between the three highest dosages rests on the following bases: (1) that the gonadotrophic complex is made up of at least two hormones; a luteinizing hormone (LH) and a follicle stimulating hormone (FSH) (Hisaw, 1947); (2) that there is an interaction between FSH and LH in causing follicular growth and ovulation; and (3) that progesterone has a "blocking effect" upon the action of the gonadotrophic complex upon the ovary (perhaps primarily upon the luteinizing hormone).

This blocking effect of the hormone should increase as the dosage is increased. When 12.5 mg. of the hormone was given per day it did not depress the luteinizing activity enough but what it could interact with FSH to bring on follicular development and ovulation. When the

dosage was increased to 100 mg. per day the gonadotrophic activity (interacting LH and FSH) was depressed to the point where the ovaries were under no more gonadotrophic stimulation than that found during the luteal phase of the estrual cycle in the untreated animal. When the treatment was stopped the endocrine system would function the same as when the animal comes to the end of the luteal phase in a normal estrual cycle.

If the blocking effects of the 25 or 50 mg. dosages were not complete then there should be marked follicular development during the course of treatment and it would not be limited to the post-treatment period. Observational laparotomies were made at the end of treatment to test this assumption on one gilt receiving the 25 mg. dose and on three gilts receiving the 50 mg. dose. Also three gilts on each of the two dosages were slaughtered 24 hours after receiving the last injection. Follicles 15 mm. or more in diameter were observed at the end of treatment in all these animals on both dosages.

If the supposition is made that 50 mg. should be a more effective "blocking" dosage than 25 mg., then an explanation must be brought forth for the greater disruption of the hormone system in the 50 mg. group than in the 25, as indicated by an absence of post-treatment estrus and the presence of cystic follicles at the time of slaughter. The former group should be under the influence of a lower luteinizing hormone-level than the latter. It seems, therefore, the theory needs to be supplemented by the assumption that there is a critical "low" level of luteinizing activity in relation to the follicle stimulating activity which is responsible for the production of cystic follicles. Partial depression of the luteinizing activity such as with the 25 mg. dosage results in an overgrowth of follicles, many of which are still capable of ovulating. Further depression to the critical low level, such as with the 50 mg. dose results in an irreversible cystic state of the follicles.

In a further attempt to study the persistence of the effect of the 50 mg. dose, two gilts were treated from day-15 to day-28 and were not slaughtered until later. The first gilt was laparotomized seven days after the end of the treatment period and her ovaries were typically cystic. Twenty-six days after the end of treatment she was slaughtered and her ovaries at that time contained 17 structures ranging from 10–53 mm. in diameter and from thick-walled, highly luteinized structures to more nearly normal appearing follicles (figure 6). An examination was made of the second gilt 22 days post-treatment and here again very large ovaries with follicles in the 20–50 mm. range with some luteinization were observed.

The data from these two gilts would indicate that the 50 mg. dosage is capable of disrupting the endocrine system in such a way that the condition of cystic follicles persists for at least a month after treatment has stopped. As noted above this does not appear to be the case if the dosage level is either increased or decreased.

Summary

Forty-three gilts were used in studying the effects of four different dosages of progesterone upon ovarian function (12.5, 25.0, 50.0, and 100.0 mg. per day). The lower dosage had no apparent effect. The three higher dosages were capable of inhibiting heat and ovulation during treatment when injections were started early enough in the estrual cycle. However, post-treatment ovulation was consistently complete (all larger follicles ovulated) only when 100 mg. daily injections were made. A high percentage of the ovaries in gilts receiving 50 mg. daily became cystic. These animals showed no heat during observation periods of 7 to 26 days post-treatment.

The theory is advanced that progesterone blocks the gonadotrophic complex, perhaps chiefly LH from interacting with FSH upon the ovary. Partial inhibition may bring about an imbalance of interacting gonadotrophic hormones which produces cystic follicles.

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IN VITRO OBSERVATIONS UPON THE NATURE OF PROTEIN INFLUENCES UPON UREA UTILIZATION BY RUMEN MICROORGANISMS[1, 2]

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APPROACHING the problem of increasing urea utilization in extending protein feed supplies in ruminant nutrition, especially in the interests of improving poor-quality roughage utilization, it became desirable to learn additional facts about the nature of influences exerted by conventional proteins already present in cattle and sheep rations upon the utilization of added simple nitrogenous compounds. Numerous feeding and nitrogen balance trials (Wegner *et al.*, 1941; Rupel *et al.*, 1943; Archibald, 1943; and Hamilton *et al.*, 1948) have directly or indirectly called attention to limitations in urea utilization when the percentage of conventional ration protein exceeded a certain point (usually 12%) or when the nitrogen of the conventional protein in the ration failed to represent a certain percentage of the total ration nitrogen being fed. Other nitrogen balance experiments with sheep (Loosli and Harris, 1945; Lofgreen *et al.*, 1947) have shown methionine to exert a favorable influence upon urea utilization. *In vitro* studies (Pearson and Smith, 1943) have further suggested that certain other amino acids exert a stimulating influence upon urea nitrogen conversion into protein by rumen microorganisms. All of the above experiments, when studied together, seem to imply that in urea utilization conventional protein or certain amino acids contained therein are beneficial, when fed in limited amounts, whereas when fed in large amounts they either fail to be beneficial or the excess ammonia formed interferes or nullifies any such beneficial effects.

The purpose of this study was to further determine the nature of protein influences upon urea utilization by rumen microorganisms making use of the artificial rumen technique.

Experimental Procedure

The plan of study in these experiments was divided into three phases. The initial phase was a comparison of urea utilization in the

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[2] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

absence of conventional protein and in the presence of respective proteins having widely different amino acid compositions. One good quality protein (casein), one poor quality protein (gelatin), and one intermediate quality protein mixture (alfalfa-timothy hay) were selected for this purpose. The flasks containing no protein and the flasks containing various proteins or protein mixture were compared at two different urea levels, as shown in table 1. In each urea-utilization-comparison in the presence of various proteins, the source of energy was varied, using all cellulose at times or a mixture of half cellulose and half corn starch. These energy variables also are presented in table 1.

The second phase of the work involved urea utilization comparisons in the presence of three different levels of casein and gelatin, respectively (table 2). In these flasks the amount of urea added (450 mg.) and the amount of energy added (18 gm. cellulose) was held constant throughout this phase. A second feature in this phase of the work was a comparison of two different methods of computing urea utilization. The first or standard method was described previously (Arias *et al.*, 1951). It can be illustrated by the following formula:

$$\text{Percentage urea utilization} = \frac{\left[\begin{array}{c}\text{Initial free } NH_3 \\ + \text{ urea } NH_3 \text{ in} \\ \text{experimental flask}\end{array}\right] - \left[\begin{array}{c}\text{Final free } NH_3 \\ + \text{ final urea } NH_3 \\ \text{in experimental} \\ \text{flask}\end{array}\right]}{\text{Urea } NH_3 \text{ added initially to experimental flask}} \times 100$$

This method of computation assumes that all ammonia formed during fermentation is of urea origin and does not take into account formation of ammonia from conventional protein in measuring the degree to which urea-ammonia is utilized.

The second method of computation corrects for the ammonia formed from conventional protein in arriving at the amount of urea-ammonia utilized. This second method of computation can be illustrated by formula as follows:

$$\text{Percentage urea utilization} = \frac{\left[\left(\begin{array}{c}\text{Initial free } NH_3 \\ + \text{ urea } NH_3 \text{ in} \\ \text{experimental} \\ \text{flask}\end{array}\right) - \left(\begin{array}{c}\text{Final free } NH_3 \\ + \text{ final urea } NH_3 \\ \text{in experimental} \\ \text{flask}\end{array}\right)\right] - \left[\begin{array}{c}\text{Final free } NH_3 \\ \text{in control flask}\end{array}\right]}{\text{Urea } NH_3 \text{ added initially to experimental flask}} \times 100$$

The carrying out of this second method necessitated the use of an alternate set of flasks (designated in the formula as control flasks)

which were exactly like the regular flasks (designated in the formula as experimental flasks) except that urea additions were omitted.

The third phase of the work consisted of urea utilization measurements using the above two formulas and comparing the influence of a series of both purified proteins and a series of protein-rich feeds, respectively. The purified proteins studied (table 3) included casein, blood fibrin, lactalbumin, zein, egg albumin, and wheat gluten. The protein-rich feeds consisted of fish meal, soybean oil meal, wheat gluten, meat scraps, linseed oil meal, and cottonseed oil meal, respectively. Each of these protein materials were added daily in amounts such that their nitrogen content equalled the nitrogen content (275 mg.) of 2 gm. of casein. Their nitrogen contents in percent were as follows: casein, 13.6; blood fibrin, 14.2; lactalbumin, 11.2; zein, 14.8; egg albumin, 12.4; wheat gluten (purified), 13.4; fish meal, 10.8; soybean oil meal, 7.3; wheat gluten, 13.3; meat scraps, 10.2; linseed oil meal, 5.6; and cottonseed oil meal, 6.8. The purified proteins were obtained from a biological preparation laboratory. The protein-rich feeds were of the type commonly fed to livestock. The principal source of energy in this third phase of the work consisted of cellulose, 9 gm. per flask.

The artificial rumen technique employed in each phase of this study was similar to that described previously (Arias *et al.*, 1951). The main features of the technique included the carrying out of fermentations in series of laboratory flasks under conditions resembling the conditions present in the live animal. The flasks were inoculated with microorganisms taken from a live animal. To these flasks were added feed ingredients every 24 hours over a 4- or 5-day period somewhat similar to the manner in which animals would be fed. Materials were likewise withdrawn at periodic intervals from the artificial rumen to determine urea and cellulose utilization as well as to maintain a constant volume of fermenting material at all times. The details of this procedure were presented by Arias *et al.* (1951).

Results

Urea utilization in the first phase of this study was found to vary considerably as influenced by the presence or absence of different quality proteins in respective fermentation flasks. In each of the comparisons (first and third, table 1) urea utilization using the standard method of computation was highest in the absence of conventional proteins as compared with its utilization in the presence of casein,

TABLE 1. THE INFLUENCE OF CASEIN, GELATIN, AND PROTEINS OF HAY UPON *IN VITRO* UREA UTILIZATION

TABLE 1. THE INFLUENCE OF CASEIN, GELATIN, AND PROTEINS OF HAY UPON *IN VITRO* UREA UTILIZATION BY RUMEN MICROORGANISMS[1]

Flask description[2]	Urea utilization (mg.) 24-hour period					Height of ammonia (mg.) 24-hour period					Cellulose digestion (pct.) 24-hour period				
	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.
First protein series using 9 gm. starch and 9 gm. filter paper in each flask															
Urea, 900 mg.	325	515	554	524	479	567	427	453	455	475	16	53	46	37	38
Urea, 1350 mg.	337	543	542	496	480	819	712	563	985	770	10	43	46	25	31
Casein+450 mg. urea	136	371	432	206	286	420	241	263	197	280	44	71	79	61	64
Casein+900 mg. urea	139	489	422	270	330	686	545	659	628	629	17	76	76	65	58
Gelatin+450 mg. urea	2	274	171	328	194	420	219	304	177	280	31	68	67	43	52
Gelatin+900 mg. urea	4	118	201	311	158	668	558	707	637	642	24	66	51	40	45
Hay+450 mg. urea	134	301	277	385	274	186	153	215	175	182	50	29	25	24	32
Hay+900 mg. urea	205	233	371	424	308	412	466	618	464	490	42	22	25	24	28
Second protein series using half starch and half filter paper as energy sources[3]															
Casein+450 mg. urea	72	34	235	242	146	480	258	223	269	307	38	70	70	21	50
Casein+900 mg. urea	235	87	348	362	258	668	357	804	688	629	54	—3	53	57	40
Gelatin+450 mg. urea	95	228	344	168	209	399	274	263	186	280	38	54	55	13	40
Gelatin+900 mg. urea	147	348	341	124	240	618	341	729	642	582	35	59	52	47	48
Casein+225 mg. urea	145	184	154	206	172	239	77	118	77	128	55	91	79	81	76
Casein+450 mg. urea	198	175	139	120	159	364	201	368	348	320	83	88	93	46	77
Gelatin+225 mg. urea	99	242	136	92	142	201	206	309	313	257	29	84	82	43	59
Gelatin+450 mg. urea	159	161	155	234	177	296	206	309	313	281	38	70	61	59	57
Third protein series using all filter paper (18 gm.) as the chief energy source															
Urea, 900 mg.	308	526	554	491	470	585	536	438	298	464	27	32	51	48	40
Urea, 1350 mg.	136	572	586	476	443	843	907	721	622	773	13	20	42	32	27
Casein+450 mg. urea	—122	457	468	168	242	541	377	215	208	335	36	62	72	51	55
Casein+900 mg. urea	—118	381	581	408	313	793	712	657	567	682	22	45	70	67	50
Gelatin+450 mg. urea	—136	318	395	312	222	471	322	188	217	299	33	53	33	45	41
Gelatin+900 mg. urea	—109	408	381	427	277	701	582	694	826	701	32	56	54	53	49
Hay+450 mg. urea	245	127	598	358	332	315	195	274	234	254	59	47	52	57	54
Hay+900 mg. urea	289	281	217	81	217	550	488	626	655	580	53	66	65	43	57

[1] Urea utilization expressed as mg. per flask based upon the disappearance of ammonia equivalent.

[2] Approximately 200 mg. of nitrogen was supplied in addition to the urea by each of the protein carriers listed below, with the exception of the second gelatin-casein comparison in the second protein series which had one-half this amount.

[3] In the first casein-gelatin comparison in this series, 9 gm. of filter paper and 9 gm. of starch were added to each flask. In the second casein-gelatin comparison only one half of these amounts were added.

gelatin, or hay proteins. Differences were noted in urea utilization when making individual comparisons of flasks containing good, intermediate, and poor quality proteins. These differences followed a protein-quality sequence. However, they were relatively small when all of the urea utilization values in casein flasks (248 mg.) were compared with similar values in gelatin flasks (202 mg.). Hay protein that was considered intermediate in quality gave average urea utilization values that were intermediate between those obtained with the good and poor quality proteins.

Little or no difference was observed between the two levels of urea in otherwise comparable flasks upon the total amount of urea-ammonia utilized. Also, the forms of energy supplied in respective flasks apparently exerted little effect upon the relative influence of individual proteins upon urea utilization.

The degree to which cellulose was digested in a given flask had a definite influence upon urea utilization. As can be seen in table 1, flasks containing casein, on the average, digested more cellulose (59%) than did similar flasks containing gelatin (48%). Flasks containing no conventional protein tended to digest somewhat less cellulose than those containing either casein or gelatin.

The heights of ammonia as presented in table 1 are of importance, primarily in demonstrating the large differences existing between pairs of flasks that were treated similarly except for different amounts of urea added. Despite the differences in heights within these pairs, there was little or no difference observed in urea utilization as was pointed out earlier.

The results obtained in the second phase of this study are presented in table 2. Adding increasing amounts of casein or gelatin to a constant amount of urea resulted in decreased urea utilization based upon the first or standard method of computation. Using this method, urea utilization averaged in the low, medium, and high casein flasks 302, —162, and —453 mg., respectively. The corresponding urea values in the presence of low, medium, and high gelatin were in the same direction (296, 102, and —67 mg. respectively) but very markedly less negative at the higher gelatin levels than was the case with casein.

Computing urea utilization by the second method (table 2) in which corrections were made for the ammonia formed from conventional protein, the values obtained with low, medium, and high casein were 408, 299, and 595 mg., respectively. Similarly, using this second method of computation, the respective urea utilization values in the

TABLE 2. THE UTILIZATION OF UREA WHEN ADDED TO FLASKS AT A CONSTANT LEVEL WITH DIFFERENT AMOUNTS OF GOOD AND POOR QUALITY PROTEINS[1]

Flask description[2]	24-hour fermentation				
	1	2	3	4	Average
Urea utilization—first method calculation					
Low casein	—150	436	563	358	302
Medium casein	—416	122	—263	— 90	—162
High casein	—754	—344	—483	—231	—453
Low gelatin	—113	293	575	427	296
Medium gelatin	—349	118	173	468	102
High gelatin	—566	— 78	95	281	— 67
Urea utilization—second method calculation					
Low casein	235	471	528	397	408
Medium casein	245	318	242	390	299
High casein	249	588	649	895	595
Low gelatin	284	374	566	439	416
Medium gelatin	291	388	314	522	379
High gelatin	298	364	464	422	387
Height of Ammonia					
Low casein	565	280	42	77	241
Medium casein	718	418	412	528	519
High casein	619	694	784	703	700
Low gelatin	510	280	35	66	223
Medium gelatin	545	361	241	206	338
High gelatin	585	429	392	272	419
Cellulose Digestion					
Low casein	46	83	63	60	63
Medium casein	53	80	27	34	49
High casein	52	77	29	56	54
Low gelatin	51	62	48	60	55
Medium gelatin	54	72	34	59	54
High gelatin	61	76	53	72	65

[1] Urea utilization was expressed as mg. per flask based upon the disappearance of ammonia equivalent.

[2] 450 mg. of urea and 18 gm. of cellulose were also added to each flask each 24-hour period.

presence of gelatin were 416, 379, and 387 mg. If one averages the low, medium, and high casein values and the similar gelatin values by this second method of calculation, values are obtained that are in close agreement, 434 mg., as compared with 394 mg.

Cellulose digestion was favorable in the flask containing the low amount of casein as compared with the flask having the low amount of gelatin. This result in table 2 was essentially the same as the results presented in table 1. At higher levels of protein, however, this relationship failed to exist; in fact, cellulose digestion was superior in the two high gelatin flasks to that occurring in the two high casein flasks.

Ammonia peaks were reached in those fermentations having the highest protein levels. In general, higher peaks, approximately double, were observed in casein as compared with gelatin flasks.

The results obtained in the third and final phase of this study are presented in table 3. With respect to cellulose digestion and height of ammonia formation, average values are tabulated for the control flasks in which urea was omitted as well as the experimental flasks containing urea. Cellulose digestion was higher in the experimental flasks containing urea, averaging 66%, than in similar flasks without urea (48%). The heights of ammonia observed were in all cases considerably higher in flasks containing urea as compared to control flasks. However, in each series of control and experimental flasks, respectively, there was considerable variation.

Urea utilization using the first method of calculation, likewise showed considerable variation ranging from a negative 54 mg. (table 3) for casein to a positive, 292 mg., in the case of fish meal. Most of this variability in urea utilization in the presence of various proteins was, however, erased when urea utilization was determined by the second method of computation. By this method urea utilization in the presence of the two respective proteins that yielded the most divergent values by the first method now resulted in values quite similar (fish meal, 437 mg. compared with 473 mg. for casein). This similarity in urea utilization in the presence of respective proteins (table 3) more or less held true with this second method of computation. One possible exception was the urea value obtained in the presence of blood fibrin. This experiment was repeated with essentially the same results. No apparent explanation is available as to why this corrected urea utilization value should be low in the presence of this particular protein.

Discussion

Interpretation of the results obtained in this study can most easily be made upon the basis that the nitrogenous requirements of rumen microorganisms are relatively simple in nature, essentially involving ammonia and not involving the more complex forms of nitrogen, such

TABLE 3. UREA UTILIZATION IN THE PRESENCE OF PURIFIED PROTEINS AND PROTEIN-RICH FEEDS

Flask description[2]		Cellulose digestion pct.	Height ammonia mg.	Urea[1] utilization 1	2
	Purified Proteins				
Casein (275 mg. N)		44	212		
Casein (275 mg. N)	+450 mg. urea	54	536	—54	473
Blood fibrin (275 mg. N)		43	35		
Blood fibrin (275 mg. N)	+450 mg. urea	69	344	122	280
Lactalbumin (225 mg. N)		51	71		
Lactalbumin (225 mg. N)	+450 mg. urea	61	406	104	492
Zein (275 mg. N)		29	34		
Zein (275 mg. N)	+450 mg. urea	61	234	265	486
Egg albumin (275 mg. N)		44	173		
Egg albumin (275 mg. N)	+450 mg. urea	61	445	—31	538
Wheat gluten (275 mg. N)		57	34		
Wheat gluten (275 mg. N)	+450 mg. urea	67	353	153	343
	Protein-rich Feeds				
Fish meal (275 mg. N)		32	22		
Fish meal (275 mg. N)	+450 mg. urea	63	240	292	437
Soybean oil meal (275 mg. N)		63	29		
Soybean oil meal (275 mg. N)	+450 mg. urea	76	240	115	401
Wheat gluten (275 mg. N)		28	40		
Wheat gluten (275 mg. N)	+450 mg. urea	74	232	248	460
Meat scraps (275 mg. N)		50	53		
Meat scraps (275 mg. N)	+450 mg. urea	63	253	157	351
Linseed oil meal (275 mg. N)		60	96		
Linseed oil meal (275 mg. N)	+450 mg. urea	76	294	137	441
Cottonseed meal (275 mg. N)		67	65		
Cottonseed meal (275 mg. N)	+450 mg. urea	65	280	125	354

[1] Urea utilization was expressed as mg. per flask, based upon the disappearance of ammonia equivalent. The values in the next to the last column were computed by the Standard Method, as described under methods, whereas the values in the last column were corrected by a second formula, also described in the methods section.

[2] Each flask also received 9 gm. of cellulose each 24-hour period.

as amino acids. This conclusion is in keeping with the concept proposed earlier (Burroughs *et al.*, 1950) and (Arias *et al.*, 1951) that rumen microorganisms have three general nutrient requirements. The first relates to energy, the second to protein or its elements, such as nitrogen,

and the third requirement relates to inorganic constituents involved in enzymes or enzyme systems of rumen microorganisms.

The strongest evidence supporting the concept that the nitrogenous requirements of rumen microorganisms are no more complex than that of ammonia is the finding that during *in vitro* fermentations as much ammonia could be utilized from urea alone (table 1) as could be utilized from urea in the presence of any conventional protein tested even after corrections were made for ammonia of protein origin (table 3). Cellulose digestion was not quite as high in table 1 when no conventional protein was present as compared to when casein or gelatin was present. However, this small difference, when based on unpublished data, may well have been due to some inorganic deficiency, such as phosphorus, which was supplied by the respective proteins.

The major interest of rumen microorganisms in proteins appeared to be that of energy rather than that of ammonia. Zein which showed visible evidence of being rather insoluble during fermentation and presumably could not be used as a source of bacterial energy, was thus found incapable of supplying sufficient ammonia for efficient cellulose digestion (table 3). Also, in fermentations (table 2) in which adequate or excessive ammonia of urea origin was available, the presence of gelatin or casein resulted in large amounts of additional ammonia being formed. This additional ammonia formation, when not required physiologically, would appear to be an expression of proteins being utilized first and foremost as energy sources in which the accompanying ammonia liberated may or may not be utilized later depending upon the quantitative needs of rumen microorganisms.

Upon the above basis, a conventional protein and in some cases an amino acid (sulphur containing) could exert one or several influences upon the conversion of urea into rumen bacterial protein. The most obvious and negative influence of a protein upon urea utilization would be the ammonia from protein origin entering into direct competition with the ammonia from urea origin in fulfilling the ammonia requirements of rumen microorganisms. This competition becomes less and allows more urea utilization when the amount of protein is reduced in a ration and in a fermentation or when the particular kind of protein is less subject to attack as a bacterial energy source and thus a small producer of ammonia from protein origin.

Not all possible protein influences are harmful to urea utilization as is the ammonia producing feature of conventional protein. Based upon the conception of the three nutrient requirements of rumen microorganisms as mentioned earlier, a protein may contribute a mineral

element, such as phosphorus or sulphur needed by the microflora in the synthesis of bacterial protein. Also, minerals in a protein or protein-rich feed may stimulate urea utilization by supplying minerals needed in cellulose digestion; thus unlocking additional energy for rumen bacterial development. Finally, the energy content of the protein itself may aid cellulose digestion directly and thus urea utilization indirectly (Arias *et al.*, 1951), in a fermentation high in roughage material and low in readily available carbohydrates.

Before concluding this discussion somewhat further mention should be made in regards to methionine and the sulphur requirements of rumen microorganisms. Methionine has been conclusively shown to aid urea utilization in studies with lambs (Loosli and Harris, 1945; and Lofgreen *et al.*, 1947). More recently Garrigus *et al.* (1950) have shown a supplementary value of methionine and a lesser supplementary value of elemental sulphur in a basal low-sulphur containing ration for lambs. More work is needed in demonstrating whether elemental sulphur or other forms of inorganic sulphur can become as effective as methionine in sheep nutrition. However, the present *in vitro* studies suggest that inorganic sulphur in the form of sulphates was as effective as the methionine contents of a large number of respective proteins in aiding urea utilization by rumen microorganisms.

Summary

Three series of experiments were used in studying the nature of protein influences upon urea utilization by rumen microorganisms making use of the artificial rumen technique. Urea utilization in the first series was studied in the absence of conventional protein compared with urea utilization in the presence of respective proteins, casein and gelatin, having widely different amino acid compositions. In the second series, further comparisons were made with urea in the presence of varying respective levels of casein and gelatin, making use of two different mathematical formulas in computing urea utilization. The third series of experiments compared urea utilization as influenced by the presence of 6 purified proteins and 6 protein-rich feeds commonly fed to livestock.

The results are interpreted upon the basis that the nitrogenous requirements of rumen microorganisms are relatively simple in nature, essentially involving only ammonia and not involving the more complex forms of nitrogen, such as amino acids. In arriving at this conclusion concerning nitrogenous requirements, other requirements such

as the energy and mineral needs of rumen microorganisms are also discussed in relation to protein influences upon urea utilization.

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THE INFLUENCE OF DIFFERENT AMOUNTS AND SOURCES OF ENERGY UPON *IN VITRO* UREA UTILIZATION BY RUMEN MICROORGANISMS[1,2]

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UREA constitutes a feed source of nitrogen potentially capable of substantially extending feed supplies of conventional protein in ruminant nutrition. Feeding trials with cattle and sheep (Bartlett and Cotton, 1938; Hart *et al.*, 1939; Rupel *et al.*, 1943; Archibald, 1943; Loosli and McCay, 1943; Owen, 1943; Willett *et al.*, 1946; Briggs *et al.*, 1947, 1948) as well as earlier trials in Germany have demonstrated the usefulness of urea as a nitrogenous supplement in partially covering the protein needs of ruminants. Nitrogen balance experiments (Harris and Mitchell, 1941; Johnson *et al.*, 1942; Harris *et al.*, 1943; Briggs *et al.*, 1946; and Hamilton *et al.*, 1948) have added further information showing that limited amounts of urea can be converted into useful protein. Voltz, as early as 1919, showed that the method of conversion was through the growth of rumen microorganisms which was further demonstrated with *in vitro* techniques by Wegner *et al.* (1940) and Pearson and Smith (1944).

The needs for additional protein feeds in balancing the total livestock ration in this country are well known. These needs have been re-emphasized in recent digestion studies with cattle (Burroughs *et al.* 1949a, 1949b, 1950b), which indicated a protein need of rumen microflora in the digestion of low-grade roughages consumed by cattle. Other nutrient (mineral) needs of rumen microorganisms have also been demonstrated in cattle digestion experiments (Burroughs *et al.*, 1950c) and by the artificial rumen technique (Burroughs *et al.*, 1950a, 1950d, 1950c).

The purpose of this study was to investigate factors governing urea utilization by rumen organisms, making use of the artificial rumen technique. The experiment was motivated by the possibility of increasing the use of urea in extending protein supplies thus making it possible to use more poor-quality roughage in cattle and sheep feeding.

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[2] Published with approval of the Director of the Ohio Agricultural Experiment Station.

[3] Part of the data presented was taken from a thesis presented to the Graduate School (1950) of the Ohio State University, by Carlos Arias in partial fulfillment of the degree, Master of Science.

Experimental Procedure

The experimental approach in these studies was to determine urea utilization in glass fermentation flasks in the laboratory somewhat similar to flasks used in earlier artificial rumen studies employed in cellulose digestion (Burroughs *et al.*, 1950a). The principal difference between present and earlier studies being the use of larger sized flasks which permitted more frequent sampling in following the course of urea transformation into ammonia and its ultimate disappearance into bacterial protein.

Two water baths maintained at 39° C. were used simultaneously, each of which contained six immersed 1000 ml. Erlenmeyer fermentation flasks that were in turn connected together with appropriate tubing for purposes of continuously bubbling carbon dioxide through the respective fermentation contents. The purpose of the carbon dioxide stream was the maintenance of anaerobiosis and the stirring of the fermentation mass.

TABLE 1. MINERAL SOLUTION RESEMBLING CONCENTRATED SALIVA

Sodium phosphate, monobasic, gm.	26.25
Sodium bicarbonate, gm.	26.25
Potassium chloride, gm.	3.75
Sodium chloride, gm.	3.75
Magnesium sulphate, gm.	1.13
Calcium chloride, gm.	0.38
Distilled water, ml.	1000

Rumen contents were secured with the use of a stomach tube or through a rumen fistula from steers withheld from a high-quality alfalfa hay ration some 12 to 16 hours prior to sampling. The contents were strained through four thicknesses of a No. 50 grade cheesecloth, care being taken in keeping the liquid warm and not unduly aerated. This liquid containing rumen microorganisms was introduced into the laboratory flasks usually in 450 ml. amounts which constituted 50 percent of the total fermentation volume. To this inoculum was added 70 ml. of a mineral solution whose composition is presented in table 1. This mineral solution, when diluted in the fermentation flask contents, was calculated to give a mineral concentration approximately one-half the mineral concentration as found in sheep saliva by McDougall (1949).

The feeds added to the flasks each 24-hour-fermentation period consisted of pure cellulose, dextrose, sucrose, corn starch, cane molasses,

and ground corn cobs. These feeds were placed in varying amounts in respective flasks as described in tables 2 and 3 in studying the influence of different amounts and sources of energy upon urea utilization. The cellulose used was either ground filter paper or a product called Solka Floc, No. 40-A.[1] The corn cobs were ground through the fine screen of a Wiley laboratory mill.

Urea was added in excessive amounts (900 mg.) in each flask at the beginning of each 24-hour-fermentation period. Usually four such fermentation periods were run on consecutive days in which the respective daily additions were similar; the only difference between the first and the three succeeding fermentations being the origin of the inoculum. The inoculum in each of these three days being one-half the residue of the previous fermentation rather than originating in an animal as was the case in the starting inoculum.

Sufficient distilled water was added to each flask to make a total of 900 ml. Hydrogen ion concentration was checked in order to maintain a pH range between 6.2 and 6.8. This was accomplished by adding a 2 molar solution of sodium carbonate whenever necessary—usually not more often than every 4 or 5 hours.

Urea utilization over any given period of time in which the total nitrogen in the fermentation flask remained constant was based upon urea-ammonia disappearance. This can be illustrated as follows:

$$\text{Urea Utilization} = \frac{\left[\begin{matrix}\text{Initial free } NH_3 \\ + \text{ urea } NH_3\end{matrix}\right] - \left[\begin{matrix}\text{Final free } NH_3 \\ + \text{ final urea } NH_3\end{matrix}\right]}{\text{Urea } NH_3 \text{ added initially}} \times 100$$

The chemical determinations required in computing urea utilization by this method were free ammonia and urea ammonia. These determinations were made at periodic intervals (usually at the beginning, after 5 hours fermentation, and at the end of the 24-hour period) upon representative aliquot samples (10 to 25 ml.) taken from each of the fermentation flasks. No free ammonia escaped from the fermenting material as checked by appropriate ammonia traps inserted in the stream of carbon dioxide passing through the flasks. In computing urea ammonia utilization for a 24-hour period, mathematical corrections were made for any loss in ammonia resulting from sampling during the progress of this time interval.

Free ammonia was determined by magnesium oxide distillation using Macro-Kjeldahl distillation equipment essentially according to

[1] Solka Floc No. 40-A was a purified form of cellulose ground medium fine and produced by Brown and Company, 110 South Dearborn St., Chicago, Ill.

the official method described in A.O.A.C. (1945). Urea was determined similarly after treatment of the sample with urease to transform urea into ammonia and making necessary corrections for free ammonia not converted by the urease added.

Cellulose digestion was determined from analyses made upon 25 ml. aliquots of fermentation material at the beginning and end of each 24-hour period. The methods employed were approximately similar to those of Crampton and Maynard (1938) with modifications such that the major part of the determinations could be carried out in a 50 ml. centrifuge tube upon the wet centrifuged material. The taking of aliquot samples was facilitated by vigorous stirring, using a jet of carbon dioxide gas. Duplicate analyses routinely carried out in many of the experiments yielded results in satisfactory agreement. Interference between the taking of urea or free ammonia samples at intermediate times between the beginning and ending of 24-hour periods with the sampling for cellulose analyses was avoided by taking the former samples in liquid phases of the contents that were free from cellulose.

Results

Increasing the energy content of the fermentation medium resulted in increased urea utilization with all sources of energy tested. This was true whether the energy source was a soluble carbohydrate, such as dextrose or sucrose, or whether the carbohydrate was more complex such as cellulose or a high fiber feed, such as ground corn cobs.

The individual data for each of the energy sources tested are presented in table 2 with respect to urea utilization as well as cellulose digestion and height of ammonia during each fermentation period. In each of these determinations, considerable day to day variation existed in the values obtained in a given flask over a four-day period. Despite this variation the average values showed trends of sufficient magnitude for interpreting their biological meaning.

Adding 9 gm. of dextrose to a flask containing 9 gm. of cellulose (table 2) increased urea utilization on the average from 33 to 53 percent. Intermediate additions of 2.25 and 4.50 gm. of dextrose gave intermediate urea utilization values averaging 38 percent. Likewise, average urea utilization for respective control and high energy addition flasks were 26 compared to 47 for molasses, 29 to 37 for sucrose, and 27 compared to 48 for corn starch. Different amounts of cellulose, 2.25, 4.50, 9.00, and 18.00 gm., resulted in urea utilizations of 14, 18, 33, and 41 percent, respectively. Ground corn cobs in the following

TABLE 2. THE INFLUENCE OF INCREASING LEVELS OF ENERGY FROM DIFFERENT SOURCES UPON UREA UTILIZATION AND CELLULOSE DIGESTION BY RUMEN MICROORGANISMS IN *IN VITRO* STUDIES

Flask addition variables	Urea utilization[1] (pct.) 24-hour periods					Cellulose digestion (pct.) 24-hour periods					Height of ammonia[2] (mgs.) 24-hour periods				
	1	2	3	4	Av.	1	2	3	4	Av.	1	2	3	4	Av.
9 gm. cellulose	23	42	27	42	33	39	66	49	18	43	555	544	656	673	607
+ 2.25 gm. dextrose	40	42	30	40	38	59	80	34	38	53	470	452	619	577	529
+ 4.50 gm. dextrose	34	46	45	26	38	30	46	52	18	36	408	336	525	573	460
+ 9.00 gm. dextrose	49	66	58	41	53	14	41	27	13	24	317	343	275	494	357
9 gm. cellulose	21	44	25	13	26	41	70	50	16	44	533	555	614	706	602
+ 2.25 gm. molasses	32	44	32	33	35	49	80	61	38	57	509	514	557	636	554
+ 4.50 gm. molasses	38	43	37	39	39	53	77	69	46	61	492	446	514	586	509
+ 9.00 gm. molasses	51	49	35	53	47	50	66	40	62	55	452	391	505	525	468
9 gm. cellulose	6	44	36	29	29	32	61	48	28	42	535	577	616	652	595
+ 2.25 gm. sucrose	24	48	44	37	38	42	55	44	27	42	507	492	564	579	535
+ 4.50 gm. sucrose	35	48	51	32	41	36	45	44	13	35	492	373	518	548	483
+ 9.00 gm. sucrose	27	53	37	29	37	20	25	13	7	16	358	375	386	548	417
9 gm. cellulose	9	44	28	26	27	30	62	40	29	40	555	592	649	682	619
+ 2.25 gm. starch	17	51	34	48	38	23	59	37	37	39	557	509	555	570	548
+ 4.50 gm. starch	23	54	46	52	44	18	46	44	34	35	553	433	443	546	494
+ 9.00 gm. starch	34	55	56	47	48	16	22	27	20	21	555	402	375	417	437
2.25 gm. cellulose	0	18	7	32	14	44	79	—2	18	35	533	662	553	435	546
4.50 gm. cellulose	9	26	10	27	18	30	79	13	35	39	548	632	581	457	554
9.00 gm. cellulose	23	42	27	42	33	39	66	49	18	43	555	544	656	673	607
18.00 gm. cellulose	42	63	9	51	41	45	67	18	20	37	632	507	697	772	652
4.50 gm. corn cobs	1	16	16	19	13						555	691	724	566	634
9.00 gm. corn cobs	3	24	27	23	19						542	654	665	619	620
18.00 gm. corn cobs	15	34	40	42	33						531	568	638	612	587
36.00 gm. corn cobs	37	55	44	80	54						527	459	516	439	485

[1] Urea utilization expressed as ammonia equivalent disappearance of the amount of urea (900 mg.) introduced in each flask at the beginning of each 24-hour period.

[2] The height of ammonia formation based upon the mg. of ammonia present in the respective flasks 5 hours after the beginning of each 24-hour period.

amounts, 4.5, 9, 18, and 36 gm. gave respective urea utilization values of 13, 19, 33, and 54 percent.

It can be observed from all the above values that although urea utilization increased significantly with each source of energy added, nevertheless the increases in urea utilization were not as great as were the energy additions. This in part was due to the inhibiting effect of certain energy additions upon the digestion of cellulose present in the fermentation material. For example, in the control flask cellulose digestion was 43 percent, whereas in the flask where 9 gm. of dextrose was added (table 2) cellulose digestion was decreased to an average value of 24 percent. Similarly, the same depression in cellulose digestion was noted with 9 gm. additions of both sucrose and starch. No such decrease in cellulose digestion occurred with increasing levels of cellulose and the same was true in the case of molasses additions. Sampling difficulties encountered made cellulose determinations unsatisfactory for corn cobs.

Despite the unfavorable influence of relatively large amounts of dextrose, sucrose, or starch upon cellulose digestion, the smallest amount of dextrose (table 2) gave improved cellulose digestion as was also true in the case of molasses. The beneficial influence of molasses upon cellulose digestion involves other constituents in addition to its sugar content and will be discussed more fully in a subsequent paper. With respect to small amounts of dextrose improving cellulose digestion, it was decided to further investigate this sugar as well as sucrose and starch to determine what influence smaller levels might exert.

The results of adding 1, 2, and 3 gm. of dextrose, sucrose, and starch, respectively, to 9 gm. of cellulose are presented in table 3.

Cellulose digestion was increased significantly with 1 and 2 gm. additions of dextrose but not with 3 gm., which coincides with the results presented in table 2. Likewise, 1 gm. additions, respectively, of both sucrose and starch resulted in increased cellulose digestion but this was not the case with higher levels of 2 and 3 gm. This result would indicate that the lowest levels of starch and sucrose used in the first experiment (2.25 gm.) was too high to demonstrate a stimulating influence upon cellulose digestion. The urea utilization values increased in table 3 with the additions of starch, sucrose, and dextrose similar to the increases noted earlier.

The heights of ammonia as presented in table 2 were the amounts of free ammonia in the experimental flasks 5 hours after each fermentation was begun. Preliminary experiments indicated complete conversion of urea by this time or somewhat earlier (usually within 3 to 5

hours). As can be seen in table 2, the free ammonia peaks were in general found in those flasks having the least amount of energy. This was more in evidence with the more readily available or more soluble carbohydrates than was the case with cellulose. Since urea utilization over a 24-hour period was essentially as good with high levels of cellulose as with high levels of readily available carbohydrates, this difference reflects an earlier utilization of urea with the more readily available carbohydrates which is later overcome in the more slowly fermenting high cellulose flask.

TABLE 3. FURTHER OBSERVATIONS UPON THE INFLUENCE OF SMALL AMOUNTS OF SUGARS AND STARCH UPON *IN VITRO* CELLULOSE DIGESTION AND UREA UTILIZATION

Flask addition variables	Cellulose digestion (%) 24-hour periods				Urea utilization (%)[1] 24-hour periods			
	1	2	3	Av.	1	2	3	Av.
9 gm. cellulose	39	64	41	48	26	49	40	38
+ 1 gm. dextrose	59	77	60	65	43	57	50	50
+ 2 gm. dextrose	68	54	50	57	45	52	51	49
+ 3 gm. dextrose	35	57	50	47	42	58	54	52
9 gm. cellulose	37	69	52	53	26	52	41	40
+ 1 gm. sucrose	47	62	61	57	34	56	57	49
+ 2 gm. sucrose	33	52	59	48	34	50	62	49
+ 3 gm. sucrose	30	55	57	47	37	63	64	54
9 gm. cellulose	35	74	54	54	26	53	47	42
+ 1 gm. starch	45	73	68	62	35	56	59	50
+ 2 gm. starch	33	64	67	55	36	56	62	51
+ 3 gm. starch	38	62	65	55	42	60	70	57

[1] Urea utilization expressed as ammonia equivalent disappearance of the amount of urea (900 mg.) introduced in each flask at the beginning of each 24-hour period.

Discussion

The results obtained in this study indicate that rumen microorganisms have energy requirements and the degree to which these requirements are fulfilled has considerable influence upon their utilization of urea or other ammonia supplying compounds. This conclusion with respect to ration energy and urea utilization finds substantiating evidence in nitrogen balance experiments with lambs (Johnson *et al.*, 1942) and in growth studies with dairy heifers (Mills *et al.*, 1942).

The source of energy furnished rumen microorganisms exerts varying influences upon urea utilization. The probability exists that there is specific need for small amounts of readily available energy, such as

dextrose, sucrose, or starch when the principal energy source is cellulose, in bringing about the most efficient utilization of urea in fermentation. This need can best be explained upon the basis that rumen microorganisms are unable to obtain energy for their body functions from cellulose until the cellulose molecule is digested or reduced in size to approximately dextrose, whereupon it can be absorbed into the bacterial cell. Immediately following the subjecting of large amounts of cellulose to fermentation there is a time interval required for rumen bacteria to become attached or in close space relationships to cellulose fibers to get their cellulose digesting mechanism working such that they can secure cellulose-dextrose as available energy. During this time interval the supplying of a small amount of supplementary dextrose would be needed.

The feeding of additional dextrose or other available carbohydrate beyond a small amount, such as the intermediate level in table 2, would appear to be well utilized by rumen microorganisms in the conversion of urea into bacterial protein. This amount of readily available carbohydrate, however, does not appear to be advantageous over other sources of energy, such as cellulose, provided adequate conditions are favorable to fiber digestion. These adequate conditions favorable to cellulose digestion are quite important and involve the supplying of essential mineral constituents in the fermentation medium (Burroughs *et al.*, 1950a, 1950d, 1950e), as will be discussed further in a companion paper.

The feeding of large amounts of dextrose or readily available carbohydrate does not seem warranted in attempts to make maximum utilization of urea as a protein substitute. These substances in large amounts have been found (unpublished data) to result in fermentation conditions unfavorable to cellulose digestion. Without appreciable cellulose digestion occurring over an extended period of time, urea utilization fails to continue beyond a short initial fermentation period. When this occurs, the bacterial protein initially synthesized from urea apparently begins to be broken down, similar to any other conventional protein, even though adequate amounts of ammonia are present in the fermentation medium.

The ideal relative amounts of different sources of energy in making maximum utilization of urea as judged from these *in vitro* studies would appear to be a medium amount of readily available carbohydrate and a medium amount of fibrous material or a large amount of fibrous material and a small amount of readily available carbohydrate. In

either case, particular attention need be given to supplying those mineral constituents favorable to maximum cellulose digestion.

Summary

Six different sources of energy, dextrose, cane molasses, sucrose, starch, cellulose, and ground corn cobs, each at three respective levels, were studied in *in vitro* fermentation flasks with respect to their influence upon rumen microorganisms in converting urea nitrogen into bacterial protein. Each source of energy whether a readily available carbohydrate or a complex carbohydrate, such as cellulose, aided urea utilization provided the latter underwent digestion. It was observed that small amounts of a readily available carbohydrate aided cellulose digestion, which in turn increased urea utilization, whereas large amounts of such materials, inhibited cellulose digestion. These results are discussed with respect to the relative merits of different combinations of readily available and less available carbohydrates as sources of energy in bringing about maximum utilization of urea by rumen microorganisms.

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MINERAL INFLUENCES UPON UREA UTILIZATION AND CELLULOSE DIGESTION BY RUMEN MICROORGANISMS USING THE ARTIFICIAL RUMEN TECHNIQUE

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EXPERIMENTS with cattle (Burroughs et al., 1950b) and with sheep (Swift *et al.*, 1950) have shown that roughage digestion can be favorably altered by mineral supplementation in rations of animals possessing a rumen. Artificial rumen studies have further indicated (Burroughs *et al.*, 1950d, 1950c) that minerals exert positive influences upon rumen microbiological digestion of cellulose and cellulose-containing roughages. These mineral influences upon cellulose digestion have been explained upon the basis that minerals represent one of three general nutrient requirements of rumen microorganisms; the other two nutrients being available energy and available nitrogen, essentially ammonia.

McNaught *et al.* (1950) have shown the effects of certain metals in stimulating the utilization of non-protein nitrogen in incubated rumen liquid. Recent artificial rumen studies (Arias *et al.*, 1951, and Burroughs *et al.*, 1951) have demonstrated the respective influence of different sources of energy and protein materials upon urea utilization by rumen microorganisms.

The purpose of this study was to extend artificial rumen observations in measuring the influence of minerals upon urea utilization in conjunction with measurements upon cellulose digestion.

Plan of Experiments

Six series of fermentations were employed in this study. The principal feature of the first series was to test the influence of water extracts of dehydrated clover, rumen ingesta, and manure, respectively, upon urea utilization when the primary energy source was cellulose. These extracts were known to be high in mineral content and approximately similar materials had previously (Burroughs *et al.*, 1949) stimulated cellulose digestion. Five different levels of each of the extracts were

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TABLE 1. THE EFFECT OF WATER EXTRACTS OF CLOVER HAY, RUMEN INGESTA, AND MANURE UPON *IN VITRO* UREA UTILIZATION AND CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Flask description	24-hour periods					
	6	7	8	9	10	Av.
Urea Utilization[1]						
9 gm. filter paper	212	131	54	32	54	97
+ 20 ml. clover extract	414	423	189	369	441	367
+ 40 ml. clover extract	311	333	234	329	266	295
+ 80 ml. clover extract	378	324	234	329	302	323
+ 160 ml. clover extract	270	342	189	221	293	263
+ 320 ml. clover extract	432	297	—50	441	234	271
9 gm. filter paper	369	139	104	126	90	166
+ 20 ml. rumen extract	266	266	315	261	275	277
+ 40 ml. rumen extract	279	315	293	297	342	305
+ 80 ml. rumen extract	257	194	158	—23	311	179
+ 160 ml. rumen extract	356	311	221	279	297	293
+ 320 ml. rumen extract	441	288	207	355	347	328
9 gm. filter paper	225	185	50	54	81	119
+ 20 ml. manure extract	284	284	266	275	347	291
+ 40 ml. manure extract	320	401	275	338	297	326
+ 80 ml. manure extract	311	410	162	347	306	307
+ 160 ml. manure extract	338	284	216	266	320	285
+ 320 ml. manure extract	216	333	284	189	381	282
Cellulose Digestion[1]						
9 gm. filter paper	26	15	2	3	—3	9
+ 20 ml. clover extract	62	75	44	55	76	62
+ 40 ml. clover extract	52	56	52	63	64	57
+ 80 ml. clover extract	69	74	46	66	66	64
+ 160 ml. clover extract	45	64	58	57	65	58
+ 320 ml. clover extract	42	45	39	42	52	44
9 gm. filter paper	64	7	13	—	6	22
+ 20 ml. rumen extract	38	34	47	41	40	40
+ 40 ml. rumen extract	42	38	48	50	51	46
+ 80 ml. rumen extract	37	21	21	22	17	24
+ 160 ml. rumen extract	59	53	53	62	53	56
+ 320 ml. rumen extract	67	54	41	46	46	51
9 gm. filter paper	29	18	62	—30	1	16
+ 20 ml. manure extract	50	44	39	34	47	43
+ 40 ml. manure extract	51	45	—12	92	43	44
+ 80 ml. manure extract	57	66	54	76	74	65
+ 160 ml. manure extract	62	69	39	23	78	54
+ 320 ml. manure extract	75	79	76	73	75	76

[1] Urea utilization expressed in mg. and cellulose digestion expressed in percentage of the amount present at the beginning of each 24-hour period.

tested, as shown in table 1. Each of these extracts (autoclaved) were prepared in a manner similar to extracts described by Burroughs *et al.* (1950a).

The second fermentation series, table 2, was designed to fractionate molasses and determine whether the sugar, the mineral content, or other constituents present were primarily responsible for enhanced

TABLE 2. ACTIVE FRACTIONS IN CANE MOLASSES WHICH STIMULATE CELLULOSE DIGESTION AND UREA UTILIZATION BY RUMEN MICROORGANISMS.

Flask description	24-hour periods					
	1	2	3	4	5	Av.
Urea Utilization[1]						
9 gm. filter paper	303	329	202	301	230	272
+ 2 gm. molasses	293	513	386	408	558	432
+ sugar[2]	296	394	270	381	324	333
+ ash of molasses[2]	399	216	447	220	366	330
+ sugar and ash[2]	294	468	250	354	636	400
Cellulose Digestion[1]						
9 gm. filter paper	51	46	63	25	29	43
+ 2 gm. molasses	45	89	19	67	81	60
+ sugar[2]	34	74	24	48	38	43
+ ash of molasses[2]	71	48	67	42	56	57
+ sugar and ash[2]	46	90	40	42	74	59

[1] Urea utilization expressed in mg. and cellulose digestion expressed in percentage of the amount present at the beginning of each 24-hour period.

[2] The sugar and ash additions were made on the basis of the amount contained in 2 gm. of molasses. The sugar of the molasses was estimated at 60% and to be a 50–50 mixture of dextrose and sucrose.

cellulose digestion and improved urea utilization that was noted earlier in artificial rumen studies, (Arias *et al.*, 1951). One negative control flask containing 9 gm. of cellulose and no molasses and one positive control flask containing a similar amount of cellulose and 2 gm. of molasses were used for this purpose. Three experimental flasks were made up in which the first was similar to the negative control flask except that a 50–50 mixture of dextrose and sucrose was added in an amount (1.2 gm.) approximating the sugar content of 2 gm. of molasses. The second experimental flask contained the ash of 2 gm. of molasses, while the third experimental flask contained both the ash and estimated sugar content of 2 gm. of molasses. No information was available concerning the origin of the molasses, except that it was

obtained from a local elevator and designated as blackstrap cane feeding molasses.

The third fermentation series (table 3) compared three different amounts of molasses ash in flasks whose principle energy source was in one case cellulose; in another comparison one-half as much starch was included; and in a third comparison, equal weights of starch and cellulose were added to flasks with different amounts of molasses ash.

TABLE 3. *IN VITRO* UREA UTILIZATION AND CELLULOSE DIGESTION BY RUMEN MICROORGANISMS AS INFLUENCED BY DIFFERENT AMOUNTS OF MOLASSES ASH IN THE ABSENCE AND PRESENCE OF STARCH USING A CONSTANT AMOUNT OF CELLULOSE

Flask description	24-hour periods				
	1	2	3	4	Av.
Urea Utilization[1]					
No starch; 9 gm. filter paper	117	302	257	247	231
+ ash from 2 gm. molasses	116	381	358	327	296
+ ash from 4 gm. molasses	134	377	340	270	280
+ ash from 8 gm. molasses	98	373	283	366	280
4½ gm. starch + 9 gm. filter paper	225	437	279	373	329
+ ash from 2 gm. molasses	333	487	466	453	435
+ ash from 4 gm. molasses	248	523	478	438	422
+ ash from 8 gm. molasses	333	534	476	484	457
9 gm. starch + 9 gm. filter paper	355	483	383	313	384
+ ash from 2 gm. molasses	366	601	451	550	492
+ ash from 4 gm. molasses	410	638	442	685	544
+ ash from 8 gm. molasses	406	737	490	709	586
Cellulose Digestion[1]					
No starch; 9 gm. filter paper	16	48	47	41	38
+ ash from 2 gm. molasses	20	73	75	83	63
+ ash from 4 gm. molasses	24	74	84	84	66
+ ash from 8 gm. molasses	8	71	71	90	60
4½ gm. starch + 9 gm. filter paper	13	55	48	32	37
+ ash from 2 gm. molasses	35	77	80	68	65
+ ash from 4 gm. molasses	22	68	84	78	63
+ ash from 8 gm. molasses	39	79	85	87	73
9 gm. starch + 9 gm. filter paper	18	27	14	4	16
+ ash from 2 gm. molasses	21	59	46	44	42
+ ash from 4 gm. molasses	23	68	39	64	48
+ ash from 8 gm. molasses	25	74	60	84	62

[1] Urea utilization expressed in mg. and cellulose digestion expressed in percentage of the amount present at the beginning of each 24-hour period.

Two purposes were involved in this series. The first was to determine whether additional ash beyond that obtained from 2 gm. of molasses would be effective in flasks in which the chief energy source added was cellulose, 9 gm. The second purpose was to determine whether the addition of starch (non-cellulose energy source) to the above amount of cellulose would increase the requirement for molasses ash. This last purpose had significance from practical feeding considerations and special significance in working out the most suitable technique in later studies in comparing the potencies of other ashes from different feed sources.

In the fourth fermentation series, the primary objective was to determine whether the ashes from a good and a poor quality roughage, respectively, would stimulate cellulose digestion and urea utilization similar to that observed with molasses ash. Three levels of molasses ash (0.125, 0.250, and 0.500 gm., respectively) were selected for this purpose. The highest level (ash from 8 gm. of molasses) had previously been shown to be an amount to which further additions of molasses ash were ineffective under the experimental conditions imposed. Similar levels of ash from dehydrated clover hay (17% protein meal) and mature timothy hay (5.4% protein), respectively, were tested under identical conditions. The ash content of these two hays was clover, 11.4 percent and timothy, 3.7 percent.

The fifth fermentation series included additions of individual mineral elements in flasks at three different levels. The elements studied were iron, magnesium, phosphorus, potassium, and calcium. Each of these elements except iron was also included in the mineral mixture, resembling the mineral content of sheep saliva (see Arias *et al.*, 1951). Thus the individual amounts of these elements added were in addition to those amounts contained in the artificial saliva. These added amounts upon the basis of the total respective flask contents (900 ml.) were iron, 5, 10, 20, 40, and 80 p.p.m.; magnesium, 5, 10, 20, 40, and 80 p.p.m.; phosphorus, 200, 400, and 800 p.p.m.; potassium, 75, 150, and 300 p.p.m.; and calcium, 10, 20, 40, and 80 p.p.m. The highest levels were included for testing possible toxicity influences of excessive amounts of a given element. The salts used were C. P. grades containing the respective elements and were ferrous sulphate, magnesium sulphate, di-sodium phosphate, potassium chloride, and calcium carbonate.

The sixth fermentation series was a continuation of the fourth and fifth series in which elements yielding positive results were studied individually and in combination with one another with respect to their being the active ingredient in molasses ash. In the sixth series (table 6)

12 artificial rumens were divided into six pairs—the distinguishing feature between members of pairs being no molasses ash in one and 0.5 gm. molasses ash in the other. The first pair constituted control flasks in which no special mineral supplements were added except those present in the mineral mixture resembling saliva. In the second pair, 10 p.p.m. iron as ferrous sulphate was placed in each flask, which represented about double the amount of iron in 0.5 gm. of molasses ash. The third pair of flasks was similar to the second pair except in place of the iron additions, a phosphorus supplement was added equivalent to double the phosphorus content estimated to be present in 0.5 gm. of molasses ash. The fourth pair contained additions of both iron and phosphorus. The fifth and sixth pairs were essentially repetitions of pairs 1 and 4 except that double the amount of artificial saliva was used.

The details of the artificial rumen technique, as used in each of the respective series, were similar to those described by Arias *et al.* (1951).

Results

Water extracts from dehydrated clover meal, rumen ingesta, and manure each stimulated urea utilization and cellulose digestion. These stimulating influences became more evident in fermentations beyond the fifth day, due probably to the disappearance or diluting out of nutrient substances carried into the artificial rumens with the large volumn (450 ml.) of inoculating material. The respective urea utilization and cellulose digestion values from the sixth through the tenth day of fermentation are presented in table 1. The smallest amount of each of the extracts (20 ml.), respectively, exerted very substantially favorable influences upon both cellulose digestion and urea utilization—the favorable influences for the most part being two- to three-fold over the respective values in the control flasks. Some further favorable influences were noted with higher levels of extracts, especially with respect to cellulose digestion. These further increases, although suggestive were not always consistent with each increased level of extracts, which probably indicates they have limited biological significance at present over the values obtained with the lowest addition of extract.

The attempt to fractionate blackstrap molasses into active organic and inorganic substances beneficial to cellulose digestion and urea utilization appeared highly successful. As can be seen from table 2, the addition of sugar in an amount estimated present in 2 gm. of molasses stimulated urea utilization over the negative control flask;

the amount of stimulation was equal to approximately one-half the stimulating influence of 2 gm. of molasses. The ash of 2 gm. of molasses likewise stimulated urea utilization approximately one-half as much as that noted with the molasses. When the sugar content and ash of molasses were combined, their respective stimulating influences were additive and were approximately equal to that of the original molasses.

Cellulose digestion, as has been previously observed, was increased significantly by molasses. The ash of molasses also increased cellulose digestion essentially as much as did the molasses (table 2). Adding sugar to molasses ash resulted in about the same digestion as with molasses or with molasses ash and distinctly better digestion than the control flask or flask with only the sugar supplement addition.

The amount of molasses ash required for efficient cellulose digestion and urea utilization was shown to be dependent upon the amount of available energy in the fermentation flask rather than specifically related to the amount of cellulose present. The ash from 2 gm. of molasses (table 3) was just as effective as larger amounts in flasks whose only energy source was 9 gm. of cellulose. Flasks containing 4½ gm. of starch in addition to 9 gm. of cellulose appeared to require slightly more ash than that obtained from 2 gm. of molasses for the most efficient digestion of cellulose and urea utilization. When the starch content was increased to 9 gm. in the presence of 9 gm. of cellulose, the requirement for molasses ash was significantly increased for both urea utilization and cellulose digestion.

Similar results with different amounts of molasses ash were obtained in a further trial (table 4) in which flasks with molasses ash were used for comparative purposes in measuring the value of ashes obtained from a high-quality hay (clover) and a low-quality hay (mature timothy). These two hay-ashes when added in amounts similar to the amount of molasses ash added resulted in rather comparable cellulose digestion and urea utilization values. This more or less equality between ashes on an ash-weight basis presumably would not exist if the ashes from these three sources were compared in amounts representative of the ash content of the feeds (timothy 3.7%, clover 11.4%, and molasses 6.2%).

The results presented in table 5 suggest that iron and phosphorus, respectively, aided cellulose digestion. However, only the iron aided urea utilization. No similar stimulations were observed with individual additions of magnesium, potassium, and calcium. However, it must be remembered that these elements were already present in the mineral mixture resembling saliva.

TABLE 4. COMPARATIVE VALUE OF ASHES OF MOLASSES, MATURE TIMOTHY HAY AND IMMATURE CLOVER HAY UPON *IN VITRO* CELLULOSE DIGESTION AND UREA UTILIZATION BY RUMEN MICROORGANISMS

Flask description	24-hour periods				
	1	2	3	4	Av.
Urèa Utilization[1]					
9 gm. each filter paper and starch	221	355	356	299	308
+ 0.125 gm. molasses ash	311	470	331	630	436
+ 0.250 gm. molasses ash	370	456	256	444	382
+ 0.500 gm. molasses ash	437	421	552	777	547
9 gm. each filter paper and starch	365	397	253	320	334
+ 0.125 gm. timothy hay ash	401	399	422	564	447
+ 0.250 gm. timothy hay ash	330	479	523	493	456
+ 0.500 gm. timothy hay ash	348	518	575	597	510
9 gm. each filter paper and starch	334	333	323	246	309
+ 0.125 gm. clover hay ash	350	465	463	441	430
+ 0.250 gm. clover hay ash	365	476	458	484	446
+ 0.500 gm. clover hay ash	376	487	526	550	485
Cellulose Digestion[1]					
9 gm. each filter paper and starch	12	14	4	7	9
+ 0.125 gm. molasses ash	17	40	20	39	29
+ 0.250 gm. molasses ash	33	33	2	11	20
+ 0.500 gm. molasses ash	43	51	68	71	58
9 gm. each filter paper and starch	35	23	0	20	20
+ 0.125 gm. timothy hay ash	35	24	16	57	33
+ 0.250 gm. timothy hay ash	22	37	44	46	37
+ 0.500 gm. timothy hay ash	36	51	55	71	53
9 gm. each filter paper and starch	21	26	31	19	24
+ 0.125 gm. clover hay ash	32	43	39	59	43
+ 0.250 gm. clover hay ash	34	44	38	63	45
+ 0.500 gm. clover hay ash	34	45	26	32	34

[1] Urea utilization expressed in mg. and cellulose digestion expressed in percentage of the amount present at the beginning of each 24-hour period.

Adding iron, phosphorus, and combinations of these elements with and without molasses ash (table 6) further showed stimulating influences of these two minerals and indicated that they probably were responsible for a part but not all of the favorable influence observed with molasses ash. In the absence of molasses ash, the single addition of iron as ferrous sulphate (flask 3) to the control flask (No. 1) significantly increased cellulose digestion and urea utilization. Adding phosphorus as sodium diphosphate alone (flask 5) to the control flask

failed to stimulate the function of rumen microorganisms until the phosphorus was added in the presence of iron (flask 7). Doubling the saliva minerals (flask 9) which contained substantially more phosphorus than flask 1 as well as additional sodium, potassium, chlorine, magnesium, calcium, and sulphur also failed to increase cellulose digestion

TABLE 5. THE INFLUENCE OF SPECIFIC MINERAL ELEMENTS UPON *IN VITRO* UREA UTILIZATION AND CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Flask description	Urea utilization[1]					Cellulose digestion[1]				
	24-hour periods									
	1	2	3	4	Av.	1	2	3	4	Av.
9 gm. filter paper	108	292	261	292	238	50	76	52	43	55
+ low iron addition	108	297	216	189	202	55	75	59	56	61
+ medium iron addition	99	274	414	292	270	54	77	53	64	62
+ high iron addition	144	310	436	338	307	53	83	64	68	67
9 gm. filter paper	32	256	144	423	214	43	69	22	56	48
+ low magnesium addition	131	184	140	423	220	62	63	21	55	50
+ medium magnesium addition	135	180	153	396	216	61	72	18	55	52
+ high magnesium addition	140	184	274	360	239	46	69	44	35	48
9 gm. filter paper	23	335	222	326	227	51	68	42	60	55
+ low phosphorus addition	58	144	266	...	...	65	90	59	..	71
+ medium phosphorus addition	81	333	297	320	258	67	90	71	67	74
+ high phosphorus addition	104	324	261	324	253	69	83	88	61	75
9 gm. filter paper	14	320	252	356	236	55	85	61	67	67
+ low potassium addition	81	310	248	378	254	67	93	60	68	72
+ medium potassium addition	27	302	351	297	244	48	69	62	57	59
+ high potassium addition	45	346	243	284	230	47	86	52	49	58
9 gm. filter paper	32	351	194	297	219	54	84	48	52	59
+ low calcium addition	90	310	202	364	242	53	79	42	61	59
+ medium calcium addition	58	346	248	238	223	54	84	60	42	60
+ high calcium addition	45	356	166	292	215	60	83	41	45	57

[1] Urea utilization expressed in mg. and cellulose digestion expressed in percentage of the amount present at the beginning of each 24-hour period.

and urea utilization until iron was added (flask 11). Control flask (No. 2) containing alfalfa ash was also further stimulated by additions of iron (flask 4) and phosphorus (flasks 6 and 8). Despite the favorable influences of the presence of iron and phosphorus, molasses ash consistently increased cellulose digestion and urea utilization somewhat further.

TABLE 6. THE EFFECT OF CERTAIN MINERALS IN MOLASSES ASH WHICH STIMULATES *IN VITRO* UREA UTILIZATION AND CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Flask number and description	24-hour periods 1	2	3	4	Av.
Urea Utilization[1]					
1. No ash	327	376	351	...	351
2. Molasses ash	474	470	486	...	477
3. No ash + iron	427	418	419	...	421
4. Molasses ash + iron	515	512	504	...	510
5. No ash + phosphorus	457	366	331	337	373
6. Molasses ash + phosphorus	520	554	582	690	587
7. No ash + iron + phosphorus	437	443	614	503	499
8. Molasses ash + iron + phosphorus	581	484	578	665	577
9. No ash, saliva minerals doubled	428	364	331	257	345
10. Molasses ash, saliva minerals doubled	532	522	595	502	538
11. No ash, saliva minerals doubled + iron	544	481	479	445	487
12. Molasses ash, saliva minerals doubled + iron	570	541	577	507	549
Cellulose Digestion[1]					
1. No ash	2	4	1	0	2
2. Molasses ash	24	35	21	51	33
3. No ash + iron	19	22	14	53	27
4. Molasses ash + iron	28	51	29	63	43
5. No. ash + phosphorus	21	9	4	12	12
6. Molasses ash + phosphorus	29	55	47	67	50
7. No ash + iron + phosphorus	26	38	51	51	42
8. Molasses ash + iron + phosphorus	53	48	51	69	55
9. No ash, saliva minerals doubled	12	6	10	14	10
10. Molasses ash, saliva minerals doubled	34	45	44	49	43
11. No ash, saliva minerals doubled + iron	43	39	25	28	34
12. Molasses ash, saliva minerals doubled + iron	43	47	39	53	46

[1] Urea utilization expressed in mgs. and cellulose digestion expressed in percentage of the amount present at the beginning of each 24-hour period.

Discussion

The results obtained in this study in conjunction with results presented elsewhere (Arias *et al.*, 1951, and Burroughs *et al.*, 1951) indicate that urea utilization in ruminants, like cellulose digestion, is dependent, to a large extent, upon considerations involving the nutrient requirements of rumen microorganisms. From the practical point of

view, these results would suggest that a roughage could be utilized most efficiently when each of the nutrient requirements, energy, protein constituents essentially, ammonia, and minerals were adequately supplied. Since urea (ammonia) constitutes one of the nutrient requirements, the results further suggest maximum urea utilization in the absence of or in the presence of minimum amounts of other ammonia-producing materials, such as proteins and when the other two nutrient requirements of rumen microorganisms, namely, energy and minerals are adequately supplied.

The mineral requirements of rumen microorganisms for maximum functioning appear to be many in number. Preliminary experiments (unpublished data) upon cellulose digestion, using the artificial rumen technique, have shown needs of varying amounts for each of the elements (sodium, potassium, calcium, magnesium, phosphorus, sulphur, and chlorine) present in the artificial saliva used in this study. The finding that iron, phosphorus, and other minerals in addition to the minerals in the artificial saliva all enter into rumen bacterial physiology is in keeping with the stimulating effects obtained from the complex mineral assortment found in the ashes of plants or plant products.

The similarity of the favorable influences exerted by equal weights of the ashes of molasses, immature clover hay, and mature timothy hay is worthy of further mention. It was not surprising that the first two ashes behaved in this fashion in that good quality hays and, to some extent, molasses have long been regarded in feeding practice as valuable assets to rations of ruminants. That the ash of poor quality hay should also give an equal stimulating influence was not predicted in advance. It should be remembered, however, that the availability of the ashes from different quality hays, when fed to animals, may vary with the maturity of a plant, as does the quantity of ash present. The immature clover contained more than three times as much ash as was present in the mature timothy hay.

Molasses has been used in this country in conjunction with urea in supplying a protein supplemental feed for cattle and sheep. This practice appears justified from the standpoint of urea utilization, as judged from present results. The ash content of molasses as well as the sugar content may contribute substantially to urea utilization as well as to roughage digestion. It should be emphasized, however, that these influences are not specific with respect to molasses but are concerned with constituents present in molasses. These constituents are also present in varying degrees in other feeds consumed by ruminants.

Therefore, molasses can only aid urea utilization when the ration is inadequate either in certain minerals and/or inadequate in available energy, such as sugar or starch.

Despite the good qualities of molasses-ash in stimulating urea utilization, there is reason to believe that this ash can be improved upon either through additions of iron, phosphorus, or possibly other elements or a different distribution of the amounts of elements already present in molasses-ash. The details of these mineral influences upon rumen bacterial physiology, both as to amount and kinds of elements involved, is worthy of additional study.

Summary

Six *in vitro* fermentation series with rumen microorganisms were employed in studying the influence of minerals upon urea utilization and cellulose digestion. In the first series, five levels of water extracts from immature clover hay, rumen ingesta, and manure were evaluated. Determination of two active fractions, namely the sugar and the ash, in blackstrap cane molasses were made in the second series. The third fermentation series measured the effectiveness of different levels of molasses-ash upon rumen microorganisms in the absence and in the presence of two levels of starch, using a constant amount of cellulose. The ashes of molasses, immature clover hay, and mature timothy hay were found approximately comparable on a weight basis in stimulating fermentations in the fourth series. In the fifth and sixth series, specific mineral elements were studied alone and in the presence of molasses-ash. Phosphorus and iron were found effective in stimulating urea utilization and cellulose digestion by rumen microorganisms. Evidence was presented that other elements besides iron and phosphorus were involved in rumen bacterial physiology in addition to sodium, potassium, calcium, magnesium, chlorine, and sulphur which were routinely used in artificial saliva.

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ESTIMATION OF THE DIGESTIBILITY OF FEEDS FROM THEIR PROXIMATE COMPOSITION[1]

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IT has long been recognized that the digestibility of nutrients in feeds is related in part to chemical composition. Almost universally, however, tabulated average digestion coefficients are applied to particular samples of feeds without regard to composition. Mitchell (1942) has noted the errors of this practice, especially with respect to the effects of components removable only by fermentation. In this connection Schneider and Lucas (1950) found that 25 to 45 percent of the variance in digestibility between samples of a given feed is associated with proximate composition (excluding ash). Thus the use of adjustments which take into account the chemical composition of a particular sample will lead to substantially more precise estimates of digestibility than will the use of tabulated averages.

Equations by which the digestion coefficients of different nutrients may be estimated from composition have been presented by various authors (Axelson, 1938, 1940, 1941, 1942, 1944 and 1949; Blaxter and Mitchell, 1948; Forbes, 1950; Forbes and Garrigus, 1950 a, b; Gallup and Briggs, 1948; Jarl, 1938; Mitchell, 1942; Schneider *et al.*, 1946 and 1947; and Stohmann, 1869).

In general the published equations employ a single composition factor such as crude protein, crude fiber, or lignin as a basis for prediction. In a few instances two predictors are used. It seems reasonable to suppose, however, that actually a number of factors affect digestibility and that better estimation of digestibility would be obtained if several predictors were utilized.

The published equations generally do not take into account the particular feed which a sample represents but base predictions only on chemical composition. The authors (Schneider *et al.*, 1950) have reported that variations in proximate composition account for only about

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half of the between-feed variance in digestibility. Thus unless factors other than those of proximate composition are used as predictors, maximum predictability cannot be obtained except by taking into account the feed involved as well as the composition of the particular sample. Digestion trials in which composition has been determined with respect to factors other than the proximate nutrients are as yet relatively few. Therefore, prediction equations that are based on sufficient data must use proximate composition percentages as predictors.

In view of the deficiencies of the published equations just discussed, the present authors have extended their earlier statistical studies (Schneider and Lucas, 1950; Schneider *et al.*, 1950) to overcome them. Equations will be presented which allow the prediction of digestibilities for a variety of nutrients in almost all feeds fed to cattle and sheep and in concentrates fed to swine. The percentages of several proximate nutrients are used as predictors, and the feed involved is also taken into account.

Procedure of Study

For the present work it was assumed that the digestion coefficients of the various nutrients (and the content of total digestible nutrients) are expressible as functions of proximate composition as follows:

$$Y=\overline{Y}+b_1 (X_1-\overline{X}_1) +b_2 (X_2-\overline{X}_2) +b_3 (X_3-\overline{X}_3) +b_4 (X_4-\overline{X}_4) \quad \text{(Eq. 1)}$$

In this equation, Y is the digestion coefficient for a given nutrient (or the content of total digestible nutrients) in a particular sample of feed; X_1, X_2, X_3 and X_4 are the percentages of crude protein, crude fiber, N-free extract and fat (ether extract) in the sample; $\overline{Y}$ is the average value of the digestion coefficient (or content of total digestible nutrients) for the feed which the sample represents; $\overline{X}_1$, $\overline{X}_2$, $\overline{X}_3$, and $\overline{X}_4$ are the average percentages of crude protein, crude fiber, N-free extract and fat for the feed, and the b's are partial regression coefficients.

Ash was omitted from the equation because it does not represent any specific combination of elements. The contents of lignin and other factors which might affect digestibility were not included because data on them were available for only a relatively small number of trials.

The data employed for this study were those used for the compilations found in *Feeds of the World, Their Digestibility and Composition* (Schneider, 1947). Before analysis, the data were divided into five classes according to type of feed, as follows: hays, dry roughages other than hay, forages cut and fed green, silages, and concentrates. In each of these classes subdivision was made according to animal species,

cattle, sheep, and swine. A final subdivision was then made to distinguish between digestion coefficients determined on feeds fed alone, and those obtained by difference.

For each of the foregoing subdivisions of data there were computed regression coefficients relating the apparent digestion coefficients for organic matter, crude protein, crude fiber, N-free extract, fat and the content of total digestible nutrients to the percentage content of the several proximate nutrients (excluding ash). Two variance sources,

TABLE 1. PARTIAL REGRESSION COEFFICIENTS FOR ADJUSTING DIGESTION COEFFICIENTS AND T. D. N. TO PROXIMATE COMPOSITION OF FEEDS *FOR CATTLE*

Class of Feeds	Predictor	Predictands					
		Organic Matter	Protein	Fiber	N.-F.E.	Fat	T.D.N.
Hays	Protein %	1.053	2.396	2.822	.517	omit	1.597
	Fiber %	—.112	.101	1.453	—.299	.443	.380
	N.-F.E. %	.476	.274	1.314	.491	—.086	.896
	Fat %	omit	omit	omit	omit	10.247	omit
Dry roughages other than hays	Protein %	.629	12.126	—.306	—.825	omit	.183
	Fiber %	.598	—1.258	.285	.110	—1.871	.599
	N.-F.E. %	.226	—1.560	—.137	.481	—2.481	.509
	Fat %	omit	omit	omit	omit	3.022	omit
Forages cut and fed green	Protein %	—.228	.145	—.202	—.182	omit	.771
	Fiber %	—.731	—.833	—.599	—.746	1.940	.002
	N.-F.E. %	—.368	—2.059	—.703	—.003	.320	.271
	Fat %	omit	omit	omit	omit	4.640	omit
Silages	Protein %	1.004	3.738	.129	.934	omit	1.388
	Fiber %	—.250	.852	—.145	—.470	—.748	—.132
	N.-F.E. %	—.134	.412	—1.114	.377	—.190	.058
	Fat %	omit	omit	omit	omit	7.064	omit
Concentrates	Protein %	—.001	.187	.922	.513	omit	.238
	Fiber %	omit	—.077	5.243	.664	.984	omit
	N.-F.E. %	—.093	—.798	6.067	.202	1.903	.395
	Fat %	—.433	omit	omit	omit	1.008	1.095

between feeds and between trials within feeds were distinguished. The regression coefficients were computed from the sums of squares and products for between trials within feeds.

Initially, all four of the regression coefficients in equation 1 were estimated. The values of the coefficients so obtained often varied markedly from one set of data to another and in some cases were unreasonable. The explanation for this is that the sum of the percentage contents of crude protein, crude fiber, N-free extract and fat is essentially a constant among samples of a given feed. Thus, the effect of the four predictors is for all practical purposes the effect of any three. A reduction of the number of predictors from four to three yielded

more consistent, and, in general, reasonable values for the regression coefficients. Practically no loss in predictability resulted.

From the mathematical standpoint the particular predictor omitted is arbitrary, but it seemed advisable to make the choice on a rational basis. The percentages selected as predictors were those occurring in

TABLE 2. PARTIAL REGRESSION COEFFICIENTS FOR ADJUSTING DIGESTION COEFFICIENTS AND T. D. N. TO PROXIMATE COMPOSITION OF FEEDS *FOR SHEEP*

Class of Feeds	Predictor	Predictands					
		Organic Matter	Protein	Fiber	N.-F.E.	Fat	T.D.N.
Hays	Protein %	.721	.009	.681	.395	omit	.866
	Fiber %	—.291	—1.276	.129	—.423	—.262	—.066
	N.-F.E. %	.101	—1.372	—.310	.299	.697	.375
	Fat %	omit	omit	omit	omit	10.534	omit
Dry roughages other than hays fed alone	Protein %	2.114	5.259	2.582	1.499	omit	2.497
	Fiber %	.037	—.801	.744	—.261	—1.283	.465
	N.-F.E. %	.293	—.981	.283	.594	—1.191	.794
	Fat %	omit	omit	omit	omit	8.453	omit
Dry roughages other than hays, fed with other feeds (digestibility "by difference")	Protein %	—.908	3.620	—2.595	—.881	omit	—.249
	Fiber %	—.055	—1.120	—.681	.219	—.725	.521
	N.-F.E.	.079	.359	—.833	.602	—.570	.771
	Fat %	omit	omit	omit	omit	4.921	omit
Forages cut and fed green	Protein %	.223	.964	.092	.124	omit	.908
	Fiber %	—.527	—.174	—.364	—.559	.191	—.021
	N.-F.E. %	—.008	—.200	—.501	.183	.098	.545
	Fat %	omit	omit	omit	omit	5.061	omit
Silages fed alone	Protein %	.946	2.812	1.090	.858	omit	.932
	Fiber %	—.433	—.143	—.036	—.577	.014	—.265
	N.-F.E. %	.100	.174	—.224	.471	.616	.072
	Fat %	omit	omit	omit	omit	5.858	omit
Silages fed with other feeds (Digestibility "by difference")	Protein %	.532	2.713	—1.101	.667	omit	1.931
	Fiber %	—.482	—.456	—.045	—.770	—.280	.357
	N.-F.E. %	.230	.123	.130	.126	—.272	.786
	Fat %	omit	omit	omit	omit	5.935	omit
Concentrates	Protein %	.747	.020	.064	.997	omit	1.052
	Fiber %	omit	—.368	.557	—.059	—1.178	omit
	N.-F.E. %	.805	—.303	.340	1.207	.012	1.049
	Fat %	.828	omit	omit	omit	1.825	2.163

largest amounts and were, insofar as possible, those believed to affect the digestibility (or the total digestible nutrients) the most. As a result of these considerations, fat content was usually omitted from the prediction equations. Those instances in which a nutrient other than fat was omitted can be ascertained from tables 1, 2, and 3.

It is assumed in the above equation that the relationships of digestibility to proximate composition are linear. This assumption is true of

TABLE 3. PARTIAL REGRESSION COEFFICIENTS FOR ADJUSTING DIGESTION COEFFICIENTS AND T. D. N. TO PROXIMATE COMPOSITION OF FEEDS *FOR SWINE*

Class of Feeds	Predictor	Predictands					
		Organic Matter	Protein	Fiber	N.-F.E.	Fat	T.D.N.
Concentrates fed alone	Protein %	—.530	—1.359	—.614	—.579	omit	—.318
	Fiber %	omit	.241	3.176	—.129	—1.399	.687
	N.-F.E. %	.431	—.035	—.854	.187	1.706	1.162
	Fat %	—.064	omit	omit	omit	17.317	omit
Concentrates fed with other feeds (digestibility "by difference")	Protein %	.586	.278	.373	.446	omit	1.055
	Fiber %	omit	—2.860	—3.406	6.881	4.992	.459
	N.-F.E. %	—.161	—1.361	.505	5.138	—.299	2.053
	Fat %	.272	omit	omit	omit	1.634	omit

crude fiber and of N-free extract. It is not strictly correct if applied to widely varying composition percentages for predicting the digestibility of crude protein and of ether extract which, because of metabolic excretion in the feces, are hyperbolic functions. Nevertheless, over the usual range of composition found in any one feed, the relationships are essentially linear (see figure 1).

Results and Discussion

The regression coefficients obtained in this study are shown in tables 1, 2, and 3 for cattle, sheep, and swine, respectively. Each table is

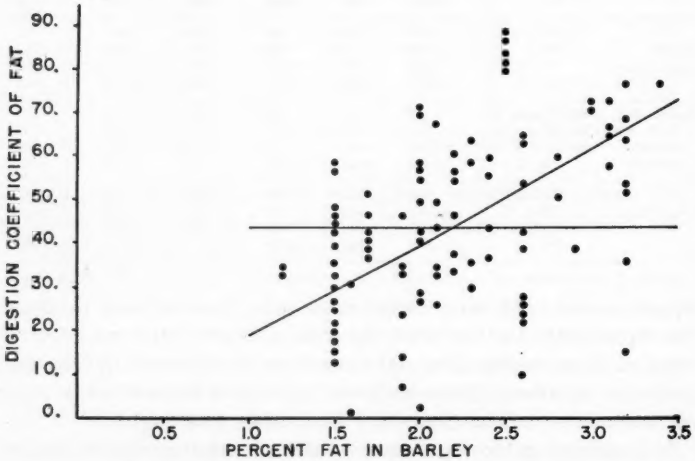

Figure 1. The relationship of fat digestibility to fat content of barley.

subdivided by class of feeds. The cases in which digestibility was determined on feeds fed alone and in which it was determined by difference are also distinguished. In each subdivision of each table a separate set of three regression coefficients is given for each of the following predictands: the apparent digestion coefficients for organic matter, crude protein, crude fiber, N-free extract (N.-F.E.) and fat (ether extract), and the percentage content of total digestible nutrients (T.D.N.). The predictands are denoted by the headings of the six columns on the right. The predictors to which the regression coefficients apply in using equation 1 are denoted in the second column from the left. The predictors are three of the four following factors expressed as percentage content in the feed on a moisture-free basis: crude protein, crude fiber, N-free extract, and fat (ether extract). The factor to be omitted is denoted by the word, "omit", in the table.

To estimate digestibility, the regression coefficients found in tables 1, 2, and 3 are substituted for the b's in equation 1. Mean values for the digestion coefficient (or total digestible nutrient content), $\overline{Y}$, and for the composition factors, the $\overline{X}$'s, are then selected from *Feeds of the World, Their Digestibility and Composition* (Schneider, 1947). The mean values selected should be those for the feed having a name which closely describes the sample. If no mean is given for the feed in question, or if the averages given for the feed are based on a small number of observations, the regression coefficients presented here should not be used. Instead, those to be given in a succeeding paper on between-feed regressions should be applied.

A computational example of the use of the values in tables 1, 2, and 3 is as follows: It is desired to estimate for cattle the digestibility of crude protein in a sample of alfalfa hay containing 16.6 percent crude protein, 26.8 percent crude fiber, 45.5 percent N-free extract, and 2.4 percent fat (ether extract) on a moisture-free basis. If one substitutes into equation 1 the mean values for "alfalfa hay, all experiments," line 2, page 171, *Feeds of the World, Their Digestibility and Composition,* and the regression coefficients for hays found in the crude protein column of table 1, the following is obtained:

$$D_{c.p.} = 70+2.396\ (16.6-15.9)+.101\ (26.8-31.7)+.274\ (45.5-41.5) = 72$$

As compared to the use of tabled average digestion coefficients without adjustment for the composition, the use of the equations presented here will result in a 25 to 45 percent decrease in the error variance with which digestion coefficients (and total digestible nutrients) are

estimated. This decrease in error warrants the use of the equations despite the computations required.

The mechanism whereby this reduction in error occurs is shown in figure 1. Data from 114 digestion trials conducted on barley with swine are plotted, illustrating the error when composition is disregarded in applying digestibility values. The sloping line represents the relationship between the fat digestibility and the fat percentage as determined from the plotted data, and the horizontal line through the data represents the overall average value for the digestion coefficient. The vertical deviations from the horizontal line are the errors incurred if one uses the average digestion coefficients without adjustment for fat content. The vertical deviations from the sloping line are the errors if adjustment for fat content is made. Obviously, on the average, the second errors are smaller than the first. In the equations presented, there is obtained an even greater reduction of error than that illustrated because adjustment is carried out for three composition factors instead of one.

The present equations yield close to the most precise estimates of digestibility which it is possible to obtain through a consideration of proximate composition. Nevertheless, as has been pointed out in earlier papers (Schneider and Lucas, 1950; Schneider *et al.*, 1950), it is logical to suppose that consideration of other chemical entities might give better predictability than is obtained here. There is thus a need that data on composition factors more basic than proximate nutrients be accumulated in connection with digestion trials.

Summary

Equations have been presented for estimating the digestion coefficients for organic matter, crude protein, crude fiber, N-free extract, fat (ether extract), and the content of total digestible nutrients in feeds, using their percentages of crude protein, crude fiber, N-free extract, and fat (ether extract) as predicting factors. Equations are given for almost all feeds which are fed to cattle and sheep, and for concentrates fed to swine. Application of the equations is illustrated and discussed. Use of the equations will result in prediction error variances which are 25 to 45 percent below those incurred if tabled average digestion coefficients are employed.

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EFFECT OF THE TIME INTERVAL BETWEEN LAST FEEDING AND SLAUGHTER UPON LEVELS OF CERTAIN B VITAMINS IN THE DIGESTIVE TRACT OF 16 WEEK OLD CALVES[1, 2]

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SYNTHESIS of B vitamins by the microorganisms within the rumen has been demonstrated by several groups, including Bechdel *et al.* (1928), McElroy and Goss (1939, 1940), Wegner *et al.* (1941), Lardinos *et al.* (1944) and Hunt *et al.* (1941, 1943). These workers allowed various periods of time to elapse between feeding and sampling of rumen contents for B vitamin analysis. Hunt (1941) noted that more thiamine was present in the rumen contents of cattle 4 hours after feeding than was present in the feed. However, after 12 to 16 hours following feeding there was a decreased content of this vitamin. Information is needed as to whether there is a peak in concentrations or whether synthesis is maintained at a steady rate, with levels of the B-vitamins remaining relatively constant. Accordingly a study was designed to investigate the effects of various time intervals following last feeding on B vitamin levels in the rumen contents of 16 week old calves. Since the contents of the entire digestive tract were available they also were studied in an attempt to follow the concentration of the vitamins throughout the entire digestive tract.

Experimental

Male Holstein calves were procured from various Pennsylvania state institutional herds and brought to an experimental barn where each animal was quartered in an individual pen. They had received colostrum for approximately 3 days. All calves received medium grade alfalfa hay *ad. lib.*, a calf starter was fed free choice up to a maximum of 5 pounds per calf per day, and each animal received a total of 300 pounds of Holstein herd milk during the first 36 days of life.

As these animals reached the age of 16 weeks they were assigned at random to groups for slaughter, two each being sacrificed at 2, 4, 6, 8

[1] Taken from data presented in a thesis to the graduate faculty of The Pennsylvania State College by E. M. Kesler in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Authorized for publication as paper No. 1651 in the Journal Series of the Pennsylvania Experiment Station.

and 12 hours after last feeding. All grain and hay was withheld during these intervals. Upon slaughter the weights of rumen contents were recorded for each calf. Following this, representative samples were collected from the contents of the rumen, omasum, abomasum, small intestine and the combined contents of the large intestine and cecum. The latter will be referred to as "large intestine." The samples were acidified, taken to the laboratory and blended into a slurry, then stored in a refrigerator until assayed.

All samples were analyzed for thiamine by the thiochrome method according to the Association of Vitamin Chemists (1947), for riboflavin by the fluorometric procedure as set forth by Loy (1947) and niacin by the cyanogen bromide-metol determination of Steele (1945). Dry matter was determined on all samples and the concentration of the three vitamins was calculated on the dry matter basis.

Representative samples of the grain and hay which the calves received were collected for assay. Concentrations of the three vitamins in these materials were determined in the same manner as were the samples of the contents. The average level of intake of each vitamin was computed for each calf. This was possible because a record had been kept of the amounts of hay and grain which the calf had eaten, and the concentration of the vitamin in the respective feedstuff had been assayed. Individual levels of vitamin intake varied in proportion to the relative amounts of the two feeds consumed.

Results and Discussion

The results of thiamine analyses are presented in table 1. From these data it may be observed that a great deal of variation existed between individual calves. In some instances vitamin concentrations were higher in the rumen and in other regions of the tract than in the feed, but other animals did not show this trend. While mean thiamine values indicate that a peak in concentrations of this vitamin occurred 6 hours after feeding, inspection of the data indicate that the difference was due almost entirely to one calf (No. 450). Analysis of variance of these data indicated that the differences between time intervals were significant at the 5 per cent level. Apparently calf 450 showed values so high that the time interval difference was significant. However, because of individual variability it is difficult to conclude that any real differences existed which could be attributed to the time intervals studied.

TABLE 1. THE EFFECT OF THE TIME INTERVAL BETWEEN LAST FEEDING AND SLAUGHTER UPON THE LEVELS OF THIAMINE IN THE DIGESTIVE TRACT OF 16 WEEK OLD CALVES
(ug/gm. of dry matter)

Time interval (hrs.)	Calf number	Level of intake	Rumen	Omasum	Abomasum	Small Intestine	Large Intestine	Mean[1]
2	454	5	5	3	2	1	1	
	459	5	9	13	11	9	4	5.8
4	455	5	8	10	5	3	2	
	456	6	7	11	5	10	6	6.7
6	450	4	22	31	17	22	18	
	451	6	5	7	4	8	2	13.6
8	452	6	8	12	6	12	12	
	453	6	8	10	9	16	11	10.4
12	457	6	8	11	5	11	7	
	458	6	6	8	4	10	4	7.4

[1] Represents the mean level of thiamine found in the digestive tract contents of the two calves slaughtered at each time interval.

As shown in table 2 there was much more evidence of riboflavin synthesis in the digestive tract of these calves than in the case of thiamine. Rumen levels of this vitamin were 180 to 390 per cent of the intake levels. With respect to the time interval the 12 hour period showed a higher mean riboflavin value than any other time. Most of this difference can be explained by the unusually high value for the small intestine sample from calf No. 458. Variations between time intervals were significant at the 5 per cent level. There was a significant difference ($P>0.01$) between regions studied, with riboflavin found,

TABLE 2. THE EFFECT OF THE TIME INTERVAL BETWEEN LAST FEEDING AND SLAUGHTER UPON THE LEVELS OF RIBOFLAVIN IN THE DIGESTIVE TRACT OF 16 WEEK OLD CALVES
(ug/gm. of dry matter)

Time interval (hrs.)	Calf number	Level of intake	Rumen	Omasum	Abomasum	Small Intestine	Large Intestine	Mean[1]
2	454	8	16	28	9	48	16	
	459	8	21	22	33	42	19	25.4
4	455	9	19	25	23	47	26	
	456	9	18	23	16	49	20	26.6
6	450	8	20	19	10	86	23	
	451	9	21	29	24	65	21	31.8
8	452	9	19	26	23	53	15	
	453	9	27	32	26	53	70	34.4
12	457	9	16	26	16	64	27	
	458	9	34	51	32	127	67	46.0

[1] Represents the mean level of riboflavin found in the digestive tract contents of the two calves slaughtered at each time interval.

in every case, in significantly higher concentration in the small intestine than in the other regions of the digestive tract.

Niacin intakes and niacin contents of the digestive tracts of these calves are presented in table 3. Large variations in the levels of this vitamin were also observed between individual calves. In only two of the ten calves were concentrations in the rumen appreciably greater

TABLE 3. THE EFFECT OF THE TIME INTERVAL BETWEEN LAST FEEDING AND SLAUGHTER UPON THE LEVELS OF NIACIN IN THE DIGESTIVE TRACT OF 16 WEEK OLD CALVES
(ug/gm. of dry matter)

Time interval (hrs.)	Calf number	Level of intake	Rumen	Omasum	Abomasum	Small Intestine	Large Intestine	Mean[1]
2	454	33	0	10	3	25	0	
	459	30	14	43	43	99	24	26.1
4	455	36	36	62	44	156	40	
	456	36	15	31	25	93	45	54.7
6	450	30	135	24	62	80	34	
	451	37	32	44	66	249	64	79.0
8	452	36	12	29	11	102	25	
	453	36	37	31	36	119	27	42.9
12	457	37	18	36	50	139	27	
	458	37	56	135	126	194	94	87.5

[1] Represents the mean level of niacin found in the digestive tract contents of the two calves slaughtered at each time interval.

than in the feed eaten, however, greatly increased levels were noted in parts of the tract other than the rumen. Concentrations of niacin were maximum 6 and 12 hours after slaughter, these mean levels being significantly greater ($P>0.01$) than those at the other intervals. The low mean value for the 8 hour interval is difficult to explain. Levels of niacin in the small intestine were much greater ($P>0.01$) than those found in the other regions of the digestive tract.

Body weight of each calf was recorded, along with the weight of rumen contents. Apparently the time interval between last feeding and slaughter was of little importance so far as rumen fill was concerned. Mean values for the weights of rumen contents were 23.7, 21.5, 23.7, 23.8 and 23.2 pounds for the 2, 4, 6, 8, and 12 hour groups respectively. No relationship was apparent between body weight and levels of the B vitamins in the rumen, or between weight of rumen contents and levels of these three dietary factors.

Summary

Ten male Holstein calves, 16 weeks of age, were slaughtered at time intervals after last feeding which varied from 2 to 12 hours. Concentra-

tions of thiamine, riboflavin and niacin were determined in the contents of the digestive tracts and compared with those in the feed eaten. Increased levels of all three vitamins were found with individual exceptions in the case of certain calves with respect to thiamine and niacin. Certain differences due to the time interval between feeding and slaughter were noted, but, due to the variability between individual animals, little importance could be attached to these differences. Therefore, under the conditions of this experiment it appeared that the time interval between last feeding and slaughter had little effect on the levels of the three vitamins in the rumen contents or in the contents of other parts of the digestive tract.

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ATROPINE BLOCKAGE OF OVULATION IN THE COW AND ITS POSSIBLE SIGNIFICANCE[1]

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IT is generally believed that ovulation in most mammalian species is brought about by a mechanism of reciprocal control by the anterior pituitary and the ovary. Follicle stimulating hormone (F.S.H.) produced by the pituitary is known to cause the growth of graafian follicles, which in turn produce estrogen. Estrogen secretion has been shown experimentally to produce a diminution of F.S.H. secretion, which is followed by the secretion of luteinizing hormone (L.H. or I.C.S.H.) L.H. brings about changes in the follicles which cause ovulation and corpus luteum formation. A third anterior pituitary hormone, prolactin or luteotropin causes the corpus luteum to secrete its hormone progesterone.

In a recent series of papers Markee, Sawyer, Everett, and others have presented evidence indicating that the nervous system is involved in the discharge of L.H. by the anterior pituitary.

Markee, Sawyer and Hollinshead (1948) found that ovulation could be induced in the rabbit by the direct instillation of adrenalin but not acetylcholine into the hypophysis. It was later discovered by Sawyer, Markee and Townsend (1949) that the release of L.H., and consequently ovulation, could be blocked in the rabbit by the administration of either atropine or the adrenolytic drug Dibenamine immediately after coitus. These results were subsequently extended to a "spontaneously" ovulating species, the rat. Sawyer, Everett and Markee (1949) found that the ovulation which normally follows the injection of estrogen on the fourth day of pregnancy in the rat could be prevented by the injection of either atropine or Dibenamine. Everett, Sawyer and Markee (1949) and Everett and Sawyer (1949) have also shown that ovulation in the cyclic rat is prevented by the injection of either atropine or Dibenamine.

It is important to know whether a neurogenic mechanism is involved in ovulation in cattle for two reasons. First, if such a mechanism exists, it may be a factor worth considering in relation to artificial insemina-

[1] Contribution from the department of animal husbandry, New York State College of Agriculture, Cornell University, Ithaca, New York.

tion techniques and second, it may give some insight into the nature of certain reproductive disturbances in the cow such as nymphomania.

Experimental Methods

Two experiments were conducted to study this problem; in the first the effect of atropine on ovulation time was determined. One estrous cycle was used as a control period in each of five heifers. During the control estrous cycle the beginning and end of estrus were determined by checking the heifer with a nymphomaniac cow at 2-hour intervals. The time of ovulation was determined by rectal palpation at 2-hour intervals. All of the palpations were performed by one of the authors (G.W.T.), who is thoroughly experienced in this technique. During a subsequent estrous cycle each of the five heifers was injected subcutaneously with atropine sulfate as quickly as possible after the beginning of estrus. The dosages used, route, and times of administration are shown in table 1. As in the control period, the animals were checked for the beginning and end of estrus at 2-hour intervals, but the rectal palpations were performed only about once every six hours after it became evident that ovulation had been blocked.

In the second experiment the beginning and end of estrus and the time of ovulation were determined as above in a control estrous cycle and in a subsequent estrous cycle during which both atropine and chorionic gonadotropin were administered as soon as possible after the heifers came in heat. The details of these injections are shown in table 2.

Some heifers were used in both experiments, but no heifer was used in the second experiment that did not experience at last two normal estrous cycles after being used in the first experiment.

In two cases in the first experiment and one case in the second experiment the information on the time of ovulation was determined by exposing the ovary to view, either by the use of a cystoscope or by the temporary installation of a plastic tube in the abdominal wall, through which a glass speculum could be inserted.

All of the heifers used were between 16 and 30 mos. of age, and their weights ranged from 590 to 1000 lbs.

Results and Discussion

The data regarding the effect of atropine administration are shown in table 1. The results are expressed in terms of hours from the beginning of estrus to ovulation, rather than from the end of estrus, because

all of the heifers but one went out of estrus within 4 hrs. after the atropine was given.

For the control periods given in tables 1 and 2 the length of the estrous period for heifers Nos. 54, 62, 63, 67, 68, 71, and 73 was 14, 7, 16, 13, 15, 12, and 14 hours, respectively, and the time elapsing from the end of estrus to ovulation was 14, 15, 9, 14, 16, 13, and 5 hrs., respectively. The average length of the estrous period was 13 hours and the average time elapsing from the end of estrus to ovulation was 12.3 hrs.

TABLE 1. THE EFFECT OF ATROPINE ON OVULATION TIME

Heifer No.	Control Period	Atropine Period			
		Treatment[1]		Time from beginning of estrus to ovulation	Ovulation Delayed
	Time from beginning of estrus to ovulation	Mg. Atropine Sulfate per Kg. body weight	Time of treatment from beginning of estrus		
	(Hrs.)		(Hrs.)	(Hrs.)	(Hrs.)
54	28	19.6	2	52.5	24.5
		15.2	5		
		4.1	7		
62	22	39.5	1	88	66
		26.3	25.5		
63	25	13.3	1	60	35
		13.3	5		
		6.7	11		
		6.7	19		
		6.7	25		
		6.7	31		
		6.7	37		
		6.7	44		
		6.7	51		
		6.7	57		
67	27	19.8	3	17	0
		13.2	6		
67	27	39.0	4	47	20
		26.0	28		
71	25	39.2	1	83	58
		26.3	25		
Av.	25.6			57.9	33.9

[1] Atropine Sulfate was dissolved in 0.9% saline at the rate of 100 mg/cc. and injected subcutaneously.

It may be seen from table 1 that, with one exception, ovulation was delayed for considerable periods of time by the administration of atropine. The average delay in ovulation due to atropine administration was 33.9 hrs.

There is no obvious explanation for the one failure (heifer No. 67), although the initial dose of atropine given was somewhat less than that given most of the other heifers. It was noted that this heifer had an unusually large follicle at the beginning of estrus. In a subsequent trial with the same heifer ovulation was delayed by the administration of a larger dose of atropine, but the delay was not as great as in the other cases.

In general, ovulation occurred when the severity of the atropine symptoms had noticeably diminished. However, it has not yet been determined whether ovulation may be prevented indefinitely by atropine.

The symptoms that occurred following the injection of these relatively large doses of atropine were striking. The pupils became extremely dilated, and the cornea became clouded so that almost complete blindness resulted. The heart and respiration rates increased. Most of the heifers became quite excitable and were likely to kick when approached. The feces became extremely hard and dry. Photophobia and ataxia were marked in all cases. Frequent, unsuccessful attempts at urination were noted in every case. Despite the severity of these symptoms, all the heifers recovered and appeared nearly normal in about two weeks time. The difficulty in urination persisted for a longer period of time.

One of the 5 heifers treated with atropine returned to estrus on the third day after ovulation. A corpus luteum was not formed after the delayed ovulation. A second ovulation occurred 7 hours after the end of the second estrus. As in the previous case, a corpus luteum was not formed, and the heifer returned to estrus 7 days later, having a very large cyst on the right ovary. This cyst was ruptured during the examination, after which a corpus luteum was formed and subsequent estrous cycles were normal. It may be worth noting that ovulation was delayed longer by the atropine in this heifer than in any of the others.

Two additional heifers acted as though they were coming in heat on the third day following the delayed ovulation, but they never reached the point where they would stand when mounted by other cows. Corpora lutea were formed in the ovaries of these 2 heifers, and subsequent estrous cycles were normal. The remaining 2 heifers showed no inclination to return to estrus; corpora lutea were formed, and subsequent estrous cycles were normal.

The second experiment was conducted in order to ascertain whether the results obtained in the first experiment were due to atropine blockage of the release of L.H. from the pituitary, or to some non-specific effect of atropine on the ovary. If it is assumed that the results of the first experiment were due to atropine blockage of some neurogenic

TABLE 2. THE EFFECT OF THE SIMULTANEOUS ADMINISTRATION OF ATROPINE AND CHORIONIC GONADOTROPIN ON OVULATION TIME

	Control Period	Atropine Plus Chorionic Gonadotropin Period			
		Treatment[a]			
Heifer No.	Time from beginning of estrus to ovulation	Mg. Atropine per Kg. body weight	Chorionic Gonadotropin	Time of treatment from beginning of estrus	Time from beginning of estrus to ovulation
	(Hrs.)		(I.U.)	(Hrs.)	(Hrs.)
54	28	39.2	1000[b] 4000[c]	2	20
62	22	39.5	1000[b] 2000[c]	2 2	
		26.3	2000[c] 4000[b]	6 32	72
67	27	39.0	1000[b] 4000[c]	1	14
68	31	39.0	1000[b] 4000[c]	1	24
73	19	32.4	1000[b] 4000[c]	5	9
Av.	(4) 26.2				16.7
Av.	(5) 25.4				27.8

[a] Atropine sulfate was dissolved in 0.9% saline at the rate of 100 mg/cc. and injected subcutaneously.
[b] Intravenously in aqueous solution.
[c] Subcutaneously in aqueous solution.

mechanism in the release of L.H., then it should be possible to produce ovulation at the normal time by the simultaneous administration of atropine and L.H. Human chorionic gonadotropin was used as a source of L.H., since it very closely resembles L.H. in all of its actions. There are, however, some distinguishing features between the two.

The results obtained by the simultaneous administration of atropine and chorionic gonadotropin are shown in table 2. With one exception (heifer No. 62) ovulation occurred slightly earlier than the normal time.

These results indicate the atropine blocks some neural mechanism involved in the release of L.H. in the bovine and that the results of the first experiment were not due to a non-specific effect of atropine on the ovary.

The failure of chorionic gonadotropin to produce ovulation at the expected time in the case of heifer No. 62 cannot be explained, but the fact that this heifer had an unusually small follicle at the time she came in estrus may be significant. In addition, the initial dose of chorionc gonadotropin given was slightly lower than that given the other cows.

In all cases corpora lutea were formed following ovulation in the second experiment.

These results indicate that a neural factor having a cholinergic component is involved in the release of L.H. from the hypophysis and ovulation in dairy cattle.

The nature of the exteroceptive pathways which may be concerned in this L.H. release mechanism and the effects of the ovarian hormones on it are being studied. It is not known whether atropine exerts its blocking effect as a result of its peripheral actions or as a result of its action on the central nervous system. The findings of Marion *et al.* (1950) are of interest in this respect. These workers found that the time elapsing from the end of estrus to ovulation was 2.18 hours shorter when heifers were served by a vasectomized bull than when service was not allowed. Similar data are being obtained for mature cows in the Cornell herd.

Summary

The time of ovulation was determined for 5 heifers in a control estrous period and in a subsequent estrous period in which atropine was administered at the beginning of heat. Ovulation was delayed from 24–66 hrs. in 4 of the 5 heifers by the atropine treatment.

The time of ovulation was also determined for 5 heifers in a control estrous period and a subsequent estrous period in which both atropine and chorionic gonadotropin, as a source of L.H., were administered at the beginning of heat. Ovulation occurred slightly earlier than the expected time in 4 of the 5 heifers treated with both atropine and chorionic gonadotropin.

These results indicate that a neural factor is involved in the release of L.H. and ovulation in dairy cattle.

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THE RELATIONSHIP OF FEED EFFICIENCY TO DIGESTION RATES OF BEEF CATTLE[1]

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ACCORDING to Schneider (1947), about 11,000 digestion trials, which have been run on the five commonest species of mammalian farm animals, have been reported in the literature. A considerable portion of this great mass of work has been done with cattle. As a result, the various factors which affect digestibility of feeds have been rather thoroughly investigated. It has been shown experimentally that digestion is affected by the kind of feed, amount of feed given and the proportion of the different feeds in the ration. These facts were shown in early tests conducted by Mumford *et al.* (1914) and Armsby (1922).

There has been considerable variation between digestion coefficients of cattle of the same breed and sex on a given ration. Experiments by Harrington (1891), Armsby (1897, 1898), Holter and Fields (1899), Hopkins (1900), Snyder and Hummel (1903), Mumford *et al.* (1914) have shown that variations between cattle on the same test may be as high as 8 to 9 percent, and even higher in isolated cases. On the average, however, variations will not be so wide. According to Morrison (1948), although individual animals may vary considerably in their ability to digest the same ration, ordinarily the variation will not be greater than 3 to 4 percent.

Since this variation exists, it was decided to see, in this trial, whether this variation in digestion rates was in any way related to feed efficiency or rate of gain of the individual animals.

Experimental

Ten steer calves of weaning age, consisting of nine Herefords and one Abeerdeen Angus X Shorthorn cross-bred, were used in this test. They were allowed the freedom of a small pen located on the south side of the barn in which they were fed. Shade was provided during the hot summer months toward the end of the trial. Twice daily the steers were placed in individual stalls for feeding. Individual feeds

[1] This work was done under R-M 714, a state contributing project to the Southern Regional Beef Cattle Breeding Program S-10 and in cooperation with the Bureau of Animal Industry, U.S.D.A.

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were placed in the open box troughs twice daily and accurate feed consumption records were kept. The steers were weighed every 28 days.

Rations

For the first 222 days of the test a growing ration, ration I, shown in table 1, was fed to the steers. A fattening ration, ration II, also shown in table 1, was fed during the last 77 days of the trial. The analysis of these rations are also shown in table 1.

TABLE 1. COMPOSITIONS OF RATIONS FED

Ingredients	Ration I	Ration II
Ground milo	20%	30%
Ground oats	15	15
Cottonseed meal	15	15
Alfalfa hay	24	24
Cottonseed hulls	24	14
Steamed bonemeal	1	1
Salt	1	1
	100	100
Analyses of Rations Fed		
Total dry matter	87.10%	81.65%
Crude protein	16.18	15.06
Ether extract (crude fat)	2.35	2.31
Crude fiber	22.25	17.24
Nitrogen-free extract	53.44	60.84
Ash	5.78	4.55

Feed and fece collections were made at two different stages of the feeding period—the growing period—referred to as trial I; the fattening period—referred to as trial II. The collection periods were one week in length.

Fecal samples were collected by use of canvas bags as designed by Garrigus and Rusk (1939). The bags, held in place by a light harness, were changed once daily during the first collection period. An increased amount of fecal excretion necessitated changing them twice daily during the latter collection period. Aliquots of ten percent of the daily excretions were placed in air-tight friction top cans and kept at a temperature of 38° F. until the end of the collection period. At that time the aliquots were thoroughly mixed and representative samples taken. These samples, of sufficient size to permit a complete routine feed analysis, were dried in flat pans in an electric oven at 105° C. for

twenty-four hours and the percentage moisture and dry matter calculated. The dried samples were then finely ground in a Wiley mill. A standard feed analysis as outlined by the Association of Official Agricultural Chemists (1945) was made.

Care was taken to collect representative feed samples during trial periods and a standard analysis was made on them.

Statistical

Digestion coefficients were determined by difference between the total intakes and total excretions of the various food nutrients for both trial I and trial II. Correlation coefficients (method of Snedecor 1946) were determined for the digestion coefficients from both trials with feed efficiency and rate of gain for the 299 day feeding period.

Results and Discussion

The average daily gains and feed efficiency, i.e. pounds of feed per pound of gain, of the individual steers for the 299-day feeding period are presented in table 2. The correlation coefficient between the daily gains and feed efficiency values is also shown in this table. The correlation coefficient of —0.64, in this case, is significant at the .05 level, indicating that daily gains and feed efficiency are inversely related. In other words, the more rapid gaining animal requires less feed per pound of gain.

Correlation coefficients between digestion rates and feed efficiency, and between digestion rates and rate of gain from trial I are also presented in table 2. It may be noted that the negative correlation coefficient of —0.68 between pounds of feed per pound of gain and digestion of crude fiber was significant at the .05 level. Correlations between feed efficiency and digestion of total dry matter, of nitrogen-free extract, crude protein and ether extract, were not significant.

Further data presented in table 2 indicates the correlation coefficients between the digestion rates and daily gains made by the steers over the 299 day period, as determined by trial I. It may be noted that none of these correlation coefficients is significant. The correlation coefficients of —0.43 in the case of crude protein and of 0.40 for crude fiber appear to be high, but due to the small number of degrees of freedom, the figures are not significant.

Table 3 contains the correlation coefficients obtained in trial II between digestion rates and feed efficiency and between digestion rates and average daily gains for the 299 day feeding period. None of these

TABLE 2. TRIAL I—AVERAGE DAILY GAIN, FEED EFFICIENCY, DIGESTION AND CORRELATION COEFFICIENTS

Steer No.	1	2	3	4	5	6	7	8	9	10[1]	Av.	St. Dev.	r Dig/Gain	r Dig/Feed Eff.
Dry matter % digested	58.06	61.10	58.04	59.24	60.85	58.90	63.82	61.91	54.89	55.39	59.22	2.80	—.07	—.16
Crude protein % digested	59.35	61.21	58.10	59.82	58.63	57.31	61.03	57.48	57.32	57.02	58.73	1.56	—.43	.16
Ether extract % digested	50.94	63.21	54.86	58.51	56.66	32.28	52.84	40.52	44.85	56.81	51.15	9.35	—.19	.44
Crude fiber % digested	28.26	36.43	39.41	43.12	43.93	42.50	43.19	45.08	38.44	30.33	39.07	5.83	.40	—.68*
NFE % digested	74.72	73.45	70.57	68.08	74.85	73.22	75.97	73.29	70.49	67.04	72.17	2.99	—.26	—.22
Daily Gain 299 days	1.34	1.37	1.75	1.67	1.82	1.87	1.70	1.54	1.54	1.82	1.64	.18		r Gain/Feed Eff.
Lbs. Feed/Lb. Gain	9.88	9.67	8.96	9.22	8.74	8.35	9.01	9.57	9.08	9.87	9.23	.51		—0.64*

[1] Crossbred Steer.
* Significant at .05 level.

TABLE 3. TRIAL II—AVERAGE DAILY GAIN, FEED EFFICIENCY, DIGESTION AND CORRELATION COEFFICIENTS

Steer No.	1	2	3	4	5	6	7	8	9	10[1]	Av.	St. Dev.	r Dig/Gain	r Dig/Feed Eff.
Dry matter % digested	60.11	63.40	62.49	65.94	65.27	59.79	67.15	63.44	60.77	60.13	62.85	2.65	.08	—.18
Crude protein % digested	57.71	63.19	58.11	61.75	59.79	56.84	63.75	59.96	57.75	58.05	59.69	2.45	—.24	.14
Ether extract % digested	58.37	58.11	63.94	61.81	60.51	54.29	62.31	60.10	59.97	43.26	58.27	5.90	—.26	—.35
Crude fiber % digested	36.85	26.66	34.09	32.60	40.18	38.44	37.58	25.36	22.99	16.96	31.17	7.75	.18	—.60
NFE % digested	72.91	79.35	77.62	80.24	76.85	74.77	79.87	78.81	79.52	77.88	77.78	2.37	.02	.03

[1] Crossbred Steer.

values is significant; however the correlation coefficient for feed efficiency and crude fiber digestion approaches significance. It may be noted from table 1 that the ration fed during trial II is lower in crude fiber than the ration fed during trial I. Hence, it is to be expected that the digestion of crude fiber in trial II would have less effect on feed efficiency. These data indicate that digestion of crude fiber is possibly one of the more important factors influencing feed efficiency.

Correlation coefficients between digestion rates and daily gains for the 299-day feeding period as determined by trial II are given in table 3. Similarly there were no significant values obtained by this analysis.

The data obtained from this investigation indicate that there is an inverse relationship between feed efficiency and rate of gain, i.e., the most rapid gaining animal requires the least feed per pound of gain. Similarly, under the conditions of this trial, feed efficiency also appears to be related to digestion of crude fiber. Animals that use the least feed per pound of gain seem to do the best job of digesting crude fiber. Since, it was demonstrated that feed efficiency is significantly correlated with rate of gain and since feed efficiency is also significantly correlated with digestion of crude fiber, it seems that the animal making the best gains would do the best job of digesting crude fiber. Due to large individual variation or to the small number of animals used, the correlation coefficient between rate of gain and digestion of crude fiber was not significant. Therefore, it is postulated that the great differences that exist between animals in their ability to make gains lies in the utilization of their food after it is absorbed rather than in differences between animals in ability to digest and absorb nutrients.

Summary

Data from ten steer calves of Hereford and of Aberdeen Angus X Shorthorn breeding were used to determine the degree of correlation between feed efficiency and digestion coefficients, between rate of gain and feed efficiency, and between rate of gain and digestion coefficients.

Data presented from one trial indicated that digestion of crude fiber was related to feed efficiency, indicating that digestion of crude fiber is possibly one of the more important factors affecting feed efficiency. In a second trial with a ration lower in crude fiber the correlation coefficient for crude fiber digestion followed a similar pattern but only approached significance. Digestion of other food nutrients did not appear to be significantly related to feed efficiency under these conditions.

Results from the two digestion trials carried out with the ten calves indicated that differences in rates of gain were probably not due to any large extent to individual differences in digestive powers of the animals.

Feed efficiency was found to be significantly correlated with rate of gain.

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THE ISOLATION OF *SPHEROPHORUS NECROPHORUS* FROM THE RUMEN TOGETHER WITH SOME FEED LOT DATA ON ABSCESS AND TELANGIECTASIS[1]

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FAIRLY general agreement exists in the literature that *S. necrophorus* is the principal bacterial agent responsible for liver abscesses. (Newsom 1938, Yamamato 1938, and Cooper, Elliot, and Woodman 1942). Smith (1944) has indicated that infection of the liver occurs by way of the hepatic portal blood, the organism gaining entry into the blood stream through ulcerative lesions or mechanical injuries of the rumen. This presupposes *S. necrophorus* to be an inhabitant of the bovine gastro-intestinal tract.

It is a common opinion of packing house and feed lot operators that long-fed feed-lot cattle exhibit a higher proportion of abscessed livers than do short fed or grass fat cattle. In one experiment, Jensen *et al.* (1947) found abscesses and "telang" to increase in the feed lot. In a subsequent experiment, however, cattle off range had 29 percent abscesses.

The work to be reported here falls into two general categories:

1. A study to determine the role of the feed lot in the development of these conditions, and
2. Attempts to produce liver damage and to isolate *S. necrophorus* from the bovine rumen.

Feed Lot Studies

Experimental Procedure

An examination was first made of records of packing houses in all areas of California. Altogether, data involving 726 lots of cattle representing 35,000 head were examined. States of origin included Arizona,

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Particular thanks are due to Harvey McDougal, W. C. Ranch Company, Collinsville, for putting the cattle from the feed lot at our disposal, and for slaughtering unprofitable animals for experimental purposes. We also are extremely grateful to James Allen, San Francisco, for making slaughtering facilities available and for rendering every assistance in these investigations.

California, Idaho, Kansas, Montana, Nevada, New Mexico, Oregon, Texas, Utah, and Wyoming. No correlation could be found between the incidence of abscesses and either the source of the cattle, location of feed lot, type of feed, or length of time on feed. Many lots of cattle off grass showed an abscess incidence as high as that observed in feed lot cattle. Condemnation reports are of limited value as they are not always accurate for individual lots and they give no idea of the condition of the abscesses, whether developing or healing, massive or small.

Several large lots of cattle of known origin were then studied. The first of these consisted of 1,600 head of 2-year-old steers fattened at the W. C. Ranch Company feed lots, Collinsville. Born in northern Arizona in the spring of 1948, they were shipped in the fall of 1949 to the Oakdale area in California, where they were run on native feed until June, 1950. They were then transferred to the feed lots. None finished on grass. On receipt, their condition was rather less than average for feeder cattle, and some difficulty was experienced in getting them on feed. Full feed consisted of 40 percent dry feed equivalent of wet beet pulp and 60 percent mill feed composed of concentrates and roughages.

On arrival at the feed lot, six head of the heaviest steers were immediately sent to slaughter. It was realized that this is an extremely small sample, but under the circumstances it was unavoidable. A further 32 head were slaughtered after 56 days and additional lots at intervals up to 160 days as shown in table 1. By this time some 1,300 head had been slaughtered and the livers of 548 examined individually for telangiectasis, "sawdust" type lesion, abscess, and fluke.

The second major lot of cattle was 160 head off irrigated pasture. Fifty-eight head were examined on slaughter right off the pasture, the remaining 102 after 100 days on a feed lot ration at Collinsville.

Finally, examination was made of the livers of 60 head of long-fed show cattle from the Grand National Livestock Exposition, San Francisco. These were yearling steers all of which had been on full feed for at least 9 months.

Experimental Results

Table 1 (a, b, c) shows the incidence of abscess, "telang", and fluke. No "sawdust" type lesions were sighted.

Abscesses.—From table 1a, it is apparent that, apart from the first sample of 6 animals, the incidence of abscesses is quite uniform, ranging from 13 to 17 percent, regardless of time in the feed lot. Although healing abscesses were present, no trend is apparent.

TABLE 1. SUMMARY OF LIVER DAMAGE IN CATTLE OF KNOWN ORIGIN

Designation of Cattle	Number of head	Days on feed	Percentage incidence						Total percentage incidence					Percentage condemnations		
			"Telang" alone	"Telang" plus abscess	"Telang" plus fluke	Abscess alone	Abscess plus fluke	Fluke alone	Normal	"Telang"		Abscess		Fluke	"Telang"	Abscess
										Very slight to slight	Moderate to marked	Active	Healing			
A. Samples from 1600 head originating in Arizona, one season pasturing in California and finished in commercial feed lot.	6	0.0	0.0	0.0	0.0	0.0	0.0	17.0	83.0	0	0	0	0	17.0	0.0	0.0
	32	56	65.6	12.5	3.1	3.1	0	3.1	12.5	56.2	25.0	15.6	0	6.2	9.0	15.6
	29	77	51.7	3.4	3.4	10.3	3.4	0	27.6	41.7	17.2	10.4	7.0	6.8	3.0	13.7
	137	112	49.0	7.3	5.8	5.1	0	7.3	25.6	51.1	11.0	11.7	0.7	13.1	3.0	12.4
	179	125	55.8	11.8	0.6	6.1	0.0	0.6	25.1	60.8	7.3	12.3	1.7	1.2	3.0	13.0
	165	160	32.5	6.1	1.2	9.7	0.6	1.2	49.7	37.6	2.4	10.9	5.4	3.0	1.0	14.0
B. 160 head of cattle, 58 of which were slaughtered directly off pasture, the remainder after 100 days in feed lot.	58	0	10.3	0.0	8.6	19.0	0.0	24.2	37.9	19	0	19.0	0	33.0	0.0	19.0
	102	100	16.7	2.9	10.8	16.7	3.9	12.7	36.2	29.4	1.0	12.7	10.8	27.0	0.0	18.0
C. Carlots of show cattle that had been fed more than 9 months.	60	>9 mo.	13.3	3.3	0.0	13.3	0.0	3.3	66.8	16.7	0	11.7	5.0	3.0	0.0	11.7

The clover-fed animals had an abscess incidence of 19 percent on entering the feed lot. After 100 days the total incidence was 23.5 percent, of which about half were healing or had healed, resulting in 18 percent being condemned. (table 1b).

Of the 60 long-fed show cattle, 7 livers were condemned for abscess. Three others had healed abscesses present, making an over-all incidence of 16.7 percent (table 1c).

Telangiectasis.—Table 1a indicates a marked increase in telangiectasis early in the feeding period, followed by a decline. Most noteworthy is the extremely high incidence of this condition, as high as 81 percent of livers examined after 56 days on feed. At this time 25 percent showed moderate to marked "telang", and 9 percent were condemned. "Moderate to marked" telang fell to 2.4 percent by 160 days and condemnations to 1 percent.

A similar trend is seen in table 1b where the sample before placing the animals in the lot was more nearly adequate. The actual increasing extent of telangiectasis after 100 days on feed was much greater than indicated in the table, as individual livers exhibited more extensive lesions although they still fell within the "very slight" to "slight" classification.

The livers of the long-fed show cattle showed very little "telang". While 16.7 percent had some telang spots, they were very slight.

No "sawdust" was sighted in any liver.

Histopathological examination was made of 41 samples of "telang" lesions, representing all stages from enlarged sinusoids to cavernous angiomata filled with normal blood and lined with a continuous endothelium. In only one case was there any sign of pressure atrophy. This exception was a liver with one large area of telangiectasis of the raised type, the hemorrhagic lesions projecting from the liver surface. Histologically this appeared as a region of enlarged blood spaces, each with a continuous endothelial lining. There was evidence of pressure in the tissue immediately surrounding the lesion, together with some necrosis and slight leucocytic invasion. Further removed from the main lesions were areas of enlarged sinusoids.

Three other cases showed some evidence of necrosis, but in none was there any of the massive necrosis reported by Getty (1946) which appears macroscopically as sawdust.

It is concluded, therefore, that the various degrees of telangiectasis sighted represent progressive stages of the condition as it occurs in this state. The primary stage is an enlargement of the blood spaces, which,

as there is no evidence of pressure *atrophy,* is probably due to active destruction of the tissue cells surrounding the sinusoids. This progresses to give large blood-filled lesions. Accompanying the destruction is regeneration of normal endothelial tissue, resulting in the ingrowth of endothelial strands and the formation of a continuous wall to the blood cavity.

Fluke.—No relationship was apparent between fluke and the other liver conditions.

Studies With S. Necrophorus

Two small experiments involving in each case 8 feeder cattle were carried out. All animals were University-bred and reared.

The objects of the first experiment were to determine on a small scale whether or not *S. necrophorus* is an inhabitant of the rumen, whether it is capable of surviving extremes of rumen environments, and finally whether drastic changes in feed consistently produce predisposing liver damage.

Consequently, the 8 steers were taken off their normal ration of alfalfa hay and limited concentrate and placed on a ration of straw and wood sulphite paper pulp. After 28 days on this ration, 4 animals were placed on a high protein concentrate mix *ad lib* with free access to alfalfa hay. At this time they were isolated into 4 groups of 2 animals each. One week after this second change in feed 2 animals on each feed regimen were inoculated into the rumen with 125 ml. of heavy broth culture of *S. necrophorus.* This was to ensure the presence of the organism in at least some of the cattle before attempting to recover it.

Rumen samples were aspirated at frequent intervals and examined for changes in pH and microflora. From 4 series of samples, the first taken prior to inoculation with *S. necrophorus* and the last 9 weeks later, attempts were made to isolate *S. necrophorus* by means of rabbit inoculation followed by culture of the ensuing abscess in thioglycolate broth. The first series of injections proved unsatisfactory in that the volume of strained rumen contents injected (2.0 ml.) was too great, and that the rabbits succumbed before the development of a large spreading abscess with localization of *S. necrophorus.* A second series of injections revealed 0.5 ml. of rumen fluid as a suitable volume, and this was used subsequently, each sample injected into 2 rabbits.

The second experiment was an attempt to induce liver abscesses by the intravenous injection of an *S. necrophorus* culture. Four steers were injected into the jugular vein, 2 with 5 ml. and 2 with 10 ml. of a thioglycolate broth culture of *S. necrophorus.* Four steers were used as

controls, all animals on a high concentrate fattening ration. Two weeks after injection, one steer was slaughtered, the remaining 3 injected animals being sacrificed one week later. At the beginning of the trial, rumen samples were taken for the isolation of *S. necrophorus*.

Results

In the first experiment, *S. necrophorus* was isolated from the rumens of 7 animals representing both nutritional states. Good duplicate results were obtained. In the second experiment, the organism was recovered from 5 steers.

Changes in rumen flora and pH following change of feed were quite consistent and comparable to those described by Van der Wath (1948). The relation between the feeding behavior of the animals and the rumen microflora was striking. Thus, following the change to straw, iodophilic bacteria disappeared entirely within a few days. Gram positive organisms also tended to disappear, but more slowly, together with a marked decrease of all types of bacteria. Following the change back to a high carbohydrate and protein ration, 2 weeks were required for the rumen flora to approximate to its original composition; and although there were no apparent digestive upsets in the cattle, this period of time was necessary for them to handle large quantities of concentrates.

After a month of semi-starvation diet, the animals given free access to the concentrate mix and alfalfa took in a heavy fill. They were still full the following day although they had stopped eating. Thirty-six hours later they began to eat again, consuming large quantities of hay. Concentrate consumption gradually increased. At ten days post change, consumption was 14 to 16 pounds daily; at 20 days, 20 pounds concentrate along with only 2 pounds of hay. The presence of 20 percent beet pulp in the concentrate mix may have modified the digestive disturbance expected from this procedure.

On the straw ration pH values read soon after collection varied between 6.9 and 7.7, with a mean of 7.3. On the fattening ration these values were 5.7 to 6.8, with a mean of 6.1. *S. necrophorus* was recovered over this full range of pH.

On slaughter, despite the drastic treatment received by these cattle, no liver abnormalities of any sort were observed.

Discussion

These results show that liver abscesses are not exclusively a feed lot problem, since cattle were observed to come off irrigated pasture with a 19 percent incidence of abscess, which did not increase in the feed

lot. The 16 percent incidence in show cattle full-fed for 9 months, with one quarter of the abscesses healed, along with the data from 1,600 head of commercially fed cattle slaughtered after varying intervals on full feed, indicate that incidence and degree of abscess development do not necessarily increase with time on feed.

The development of telangiectasis may, however, be related to metabolic upsets involved with changes in feed and rumen flora when the animals are first brought into the feed lot. That "telang" is not related to uniform high feeding over a prolonged period is indicated by decreasing incidence with time in the feed lot. The long-fed, very fat show cattle showed a very low incidence. Marked changes of feed under natural field conditions may induce "telang".

There is no evidence that abscesses and "telang" are at all related in California. While in 2 cases small, apparently developing abscesses were seen in the region of "telang" lesions, histopathological examination failed to reveal any definite correlation. Also "telang" showed a tendency to develop in one region—the top parietal portion of the liver. Abscesses did not follow this distribution. It has been suggested that areas where massive necrosis ("sawdust") is common would be ideal foci for abscess development. However, in California such necrosis is extremely rare; in fact none has been sighted. Hence, it is believed that abscess and telangiectasis should, for the present, be regarded as separate entities.

The source of infection by *S. necrophorus* is important. It can survive in the rumen under widely different nutritive states. Although we were unable either to produce "telang" by marked feed changes on a limited scale, or to show any relationship between abscess and telang, we did obtain some evidence from the lots of commercial cattle that telang increases with the marked change and increase in feed when cattle are placed in the feed lot. Such changes may also affect the lining of the rumen and other parts of the gastro-intestinal tract, permitting entry of *S. necrophorus* to the portal blood. It seems possible too that continuous exposure to the organism may be necessary. Thus we produced no liver abscesses in 4 cattle, each of which received a single large injection of an organism culture into the jugular. A constant source of infection could be foot rot or *S. necrophorus* infections elsewhere in the body.

The frequent high incidence of liver abscess in range and irrigated pasture cattle indicates the necessity of seeking further back in the origin of the cattle for source of infection and predisposing factors, if any, that result in liver abscess.

To study the occurrence or development of liver abscesses under a

given production condition such as a feed lot, large numbers are required. For example, to determine that the animals were free from abscess at the start a sample of 50 showing no abscesses would be necessary, to have odds of 19 to 1 that the incidence in the population was between 0 and 7 percent. Sampling during the course of a feeding period should be at random rather than based on condition of the cattle. Thus if the presence of abscesses tended to restrict gains and finish, cattle selected on condition would be killed after longer periods on feed. This would result probably in a spurious correlation of abscess incidence and time on feed.

Summary

1. Experiments and observations made on the problems of liver abscess and telangiectasis during 1950 are presented.

2. No apparent relation exists between time in the feed lot and the incidence of liver abscesses.

3. There appears to be an increase in telangiectasis shortly after cattle are placed on full feed in the feed lot. The incidence then appears to decrease after 50 days.

4. Telangiectasis is capable of healing without fibrosis.

5. The incidence of telangiectasis in California is very much higher than is indicated by condemnation records.

6. "Sawdust" type lesion is extremely rare in California.

7. The upper parietal region of the liver, in the area of attachment to the diaphragm, appears most susceptible to telangiectasis.

8. There is no demonstrable relationship between telangiectasis, abscess, and fluke, nor is there any evidence that abscesses are more likely to develop in areas of telangiectasis than in other areas of the liver.

9. The isolation of *S. necrophorus* from the bovine rumen is described. It is capable of surviving extremes of rumen environment.

10. This study includes also description of unsuccessful attempts to induce liver damage and *S. necrophorus* infection experimentally by drastic feed changes and injection of the organism by stomach tube and intravenously.

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WATER CONSUMPTION OF HEREFORD AND BRAHMAN CATTLE AND THE EFFECT OF COOLED DRINKING WATER IN A HOT CLIMATE[1]

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THIS is a continuation of work done in the Imperial Valley and reported by Ittner and Kelly (1948, 1950) on ways of making life more comfortable for livestock in hot climates. Shades and sprinklers have been used to cool the outside of cattle; it was decided to see what effect cooling the inside of cattle would have on their rates of gain, food and water consumption, and general comfort. This was done by cooling the drinking water by refrigeration for one group of Herefords. A check group of Herefords and a group of Brahmans had drinking water that was unchanged except as the water temperature varied normally with air temperature. Previous work by Hickman, Rinehart and Johnson (1934), and Foster (1944) has shown that the only benefit gained by warming drinking water in cold climates is the prevention of freezing. Gerlaugh (1932), Stanley (1938), and Bonsma (1939) have reported on the water requirements of livestock. Stanley reports that range cows drank a maximum of 11.5 gallons per day in summer.

The water economy of farm animals was reviewed by Leitch and Thomson (1944). The physiological background of water economy was discussed by Thompson, Worstell, and Brody (1949). This and subsequent publications of the Missouri group present data on water consumption, cardiorespiratory activities, insensible weight loss, and moisture vaporization in dairy cattle as influenced by environmental temperature (Ragsdale, Worstell, Thompson, and Brody, 1949; Kibler and Brody, 1949; and Thompson, McCraskey, and Brody, 1949).

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Materials and Methods

Two groups of Herefords, including 3 steers and 1 heifer each, averaging 840 pounds in weight, were used to measure the effect of cooling water. A third group, of 4 Brahman steers, was used to compare the consumption of warm (uncooled) water by this breed with that of Herefords. The 3 groups were placed in separate pens, each pen provided with a circular concrete water tank 30″ high and 36″ in diameter. The tank in Pen 1 was insulated with commercial insulation and the top covered with 2″ planks, except for a small opening for the animals to drink through. The water in this tank was maintained at 65°F±2° by a ¾ horsepower mechanical refrigeration unit with direct expansion coils immersed in the water. A small pump at the bottom of the tank provided agitation and kept the cold water from settling to the bottom.

The consumption of water in each of the 3 pens was measured by calibrated water meters on the water inlets. Since the accuracy of these meters is highest when a full stream of water is flowing through them, a float and micro-switch was arranged with a solenoid water valve so that full flow was obtained whenever the water level in the tank dropped 1 inch. The water meters were read at least twice a day; and on some days hourly observations were made.

The tops of the 2 uncooled tanks in Pen 2 (Herefords) and Pen 3 (Brahmans) were not covered. All 3 tanks were shaded. Shades were also provided for the animals a few feet from the tanks, the Brahmans' shade being the least effective in reducing the radiant heat load. A 2-pen recorder kept a continuous record of the temperature of the water near the top of the cold tank (Pen 1) and 1 warm tank (Pen 2). In a nearby weather station, a hygro-thermograph kept a continuous record of the air temperature and relative humidity.

The rate of water evaporation from the cold water tank was measured both before and after the summer feeding test. Evaporation from the warm water tanks was measured by comparison with an identical tank before, after, and several times during the test. An average of 2 gallons per day was lost by evaporation from the warm tanks, while only 0.4 gallon per day was lost from the cold tank. These figures were used to adjust the water consumption as given by the meters for each lot of animals.

Both lots of Herefords were given uncooled water only for 21 days (May 24-June 13) prior to the start of the feeding trial, to compare their intake of normal temperature water. On June 14, 1950, the 12

test animals were weighed, the water in Pen 1 cooled to 65°F, and the feeding experiment started. The test was run until September 5, a total of 83 days.

All groups were fed twice a day and were given all the good quality hay they would clean up between feedings. The ration consisted of 75 percent alfalfa hay and 25 percent barley hay. Every 28 days, the animals were weighed after they had been in a dry lot for 10 hours without feed or water.

Results

The mean air temperature for the test period (June 14 to September 5) was 85.4°F, the mean maximum 100.5°F, and the mean minimum 70.2°F; the lowest reading recorded was 54°F and the highest 118°F. Only 29 days of this period had minimum readings below 70°F.

There were several thunder showers and rather high humidity in July; while August, except for the last week, was dry. The reverse of this is usually the case. The average noon relative humidity for the test period was 38.4 percent and the average daily humidity 46.7 percent. The week of July 3 was the most humid, 72.2 percent while the driest week was August 7, with a humidity of 29.6 percent.

The average water temperature in Pen 2 for the period was 88.2°F. At 6 a.m., the water temperature averaged 83°F. From this time until 11 a.m., there was a rapid rise to 90°F due to the sun hitting the side of the tank. An average maximum of 94.5°F was reached at 5 p.m., the hottest part of the day, but this rise was rather gradual. The water temperature fell from 5 p.m. to 6 a.m. in almost a straight line back to 83°F. The highest and lowest water temperatures recorded in the warm tank were 97° and 72°F respectively.

While no record was obtained of the temperature of the water drunk by the Brahmans (Pen 3), it must have been similar to that of Pen 2, since the tanks were identical, with about the same exposure.

Table 1 gives the rates of gain, food consumption, and feed per 100 pounds of gain for each group of animals. The 4 Herefords receiving the cold water (Pen 1) had an average daily gain of 1.46 pounds per day; Pen 2 (warm water) was 1.07 pounds; and Pen 3 (Brahmans), 1.45 pounds of gain per day.

Analysis of variance of average daily gains is tabulated below:

	D.F.	S.S.	M.S.
Total	11	0.7978	.....
Pens	2	0.3930	0.1965*
Within pens	9	0.4048	0.0450

* indicates significance at the 5 percent level.

The 95 percent confidence limits for the difference between the Hereford groups on warm and cooled water (estimated difference, 0.39 pounds daily) are 0.05, 0.83; and those for Pen-2 Herefords and Pen-3 Brahmans (estimated differences, 0.38) are 0.04, 0.82.

Average daily water consumption per animal and the average daily water consumption per 100 pounds of live weight are shown in table 2.

TABLE 1. AVERAGE WEIGHTS, DAILY GAINS, AND FOOD CONSUMPTION OF FOUR HEREFORDS RECEIVING COLD DRINKING WATER AND FOUR HEREFORDS AND FOUR BRAHMANS RECEIVING WARM DRINKING WATER

(All figures are in pounds)

June 14–Sept. 5, 1950

	Pen 1	Pen 2	Pen 3
	Herefords (Cold water)	Herefords (Warm water)	Brahmans (Warm water)
Av. initial weight	849	833	583
Av. final weight	970	922	704
Av. daily gain	1.46	1.07	1.45
Av. daily feed			
Alfalfa hay	17.70	16.90	13.79
Barley hay	5.94	5.52	4.42
Total	23.64	22.42	18.21
Feed/100 pounds gain			
Alfalfa hay	1212	1577	950
Barley hay	406	515	305
Total	1618	2092	1255

Cooling the water to 65°F resulted in consumption of about 1.25 gallons less daily per animal compared with pen 2. During the initial period (May 24-June 13), when both Pens 1 and 2 were receiving warm water, consumption was the reverse of this, the animals in Pen 1 consuming about 1.3 gallons per day more than Pen 2. The smaller Brahmans drank less water than either of the two lots of Herefords. Even when their water consumption was calculated on the basis of gallons consumed per 100 pounds of live weight, they still had a smaller water consumption than the Herefords.

Figure 1 shows that all groups of animals drank more water on the warmer days than they did on the cooler days. This is especially true with the warm water Herefords of Pen 2. As a further check on this, the average water consumption on about 20 days with a mean air temperature of 83°F or less was compared with the average water

consumption on about 20 days with a mean air temperature of 88°F or more. In all cases, the water consumption was more on the hotter days. The differences found were: warm water Herefords, 2.23 gallons per day per head, more on the hot than on cool days; Brahmans 1.30 gallons per day; and cold water Herefords only 0.49 gallon more per head daily.

TABLE 2. AVERAGE DAILY WATER CONSUMPTION PER ANIMAL AND AVERAGE DAILY WATER CONSUMPTION PER 100 POUNDS OF LIVE WEIGHT

	Pen 1		Pen 2		Pen 3	
	Herefords Cold-water consumed, gallons daily		Herefords Warm water consumed, gallons daily		Brahmans Warm water consumed, gallons daily	
Period of Experiment	per head	per 100 pounds weight	per head	per 100 pounds weight	per head	per 100 pounds weight
Preliminary,[1] May 24–June 13	16.93	2.09	15.62	1.97	....	....
First, June 14–July 11	15.91	1.83	16.10	1.90	9.72	1.60
Second, July 12–Aug. 8	15.07	1.65	16.61	1.89	9.89	1.52
Third, Aug. 9–Sept. 5	15.62	1.64	17.72	1.95	10.68	1.60
Average: June 14–Sept. 5	15.36	1.69	16.61	1.89	9.98	1.55

[1] Both lots of Herefords drinking uncooled water.

Several sets of hourly readings of water consumption were taken throughout the test. Most of the water was consumed by the Herefords in two 4-hour periods during the day: 7 a.m. to 11 a.m., and 4 p.m. to 8 p.m. This coincides with the times of feeding, 7:30 a.m. and 5 p.m. Both groups of Herefords drank approximately 1.1 gallon per hour per head during the 4-hour morning period and 1.7 gallon per hour per head in the evening. During the heat of the day, 11 a.m. to 4 p.m., only about 0.4 gallon per hour per head was consumed; and, during the night, when the water was fairly cool, the animals drank only 0.3 gallon per hour per head. The Brahmans drank less than these amounts but followed the same trend reported above.

Four sets of respiration rates were taken on 4 separate days, between 2 and 4 p.m., when the air temperature ranged from 106°F to 116°F. Average respirations per minute for each lot were: Pen 1, 124; Pen 2, 137; and Pen 3, 77. Two sets of respiration rates were taken in the morning between 9 and 11, with the air temperature around 90°F. These averages were as follows: Pen 1, 87; Pen 2, 96; and Pen 3, 51.

The Brahmans respired fairly fast, but their respirations were much shallower than the Herefords, and they showed very little discomfort from the heat.

Discussion

Calculated on the bases of (1) average water consumption for the two lots of Hereford cattle, (2) the average temperature of the water (cooled 15.36 gallons, 65°F; uncooled 16.61 gallons, 88.2°F) and (3) an average body temperature of 103°F, (assumed from other data) the cooled water decreased the heat load by about 2800 B.t.u. or 700 Kg calories daily. Although this amount would raise about 1.9 gallons of ice water to the boiling point, it does not loom large compared with average basal heat production (about 11 percent) or as compared with

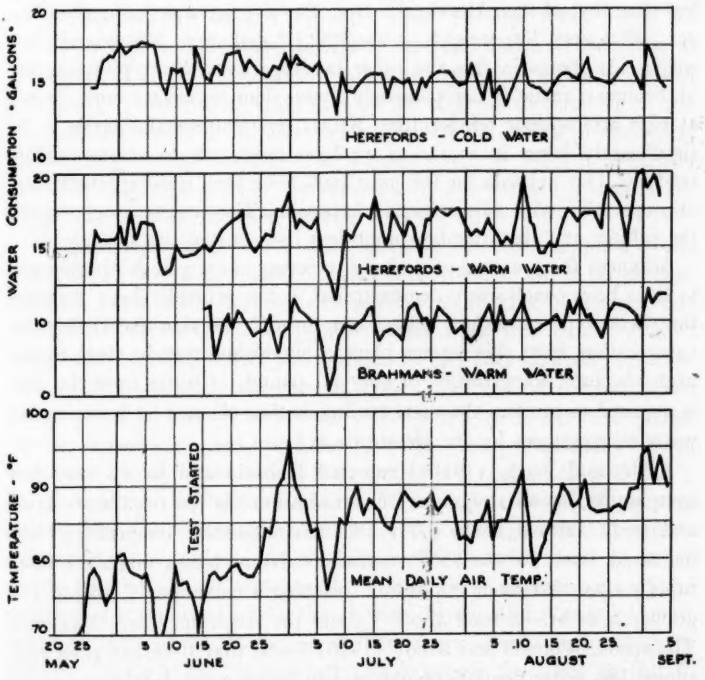

Figure 1. Relation between water consumption and mean daily air temperature for three groups of cattle at Imperial Valley Field Station, 1950.

the estimated lightening of heat load by shade (1344 B.t.u. per hour at 100°, Kelly and Ittner 1949).

The ratio of water consumed to air-dried feed eaten was 5.4 and 6.2:1 respectively for the cooled and the warm water. These figures are roughly double the observed consumption of cool water (50°±10°F) by non-lactating dairy cows under temperate conditions. (Data cited by Thompson, Worstell, and Brody 1949). Thus the high water intake and the high correlation of water intake with atmospheric temperature variations in this study confirm under field conditions the results reported by the Missouri workers as obtained under controlled conditions of their Climatic Laboratory. The total effect of the average water consumption response of the animals to high temperature (doubling of intake) can be estimated at approximately 2400 B.t.u. or 600 Kg. calories in the case of Lot 1 (cooled water).

The warm water consumption of the Brahman cattle was consistently less than that of their Hereford mates. The average was 0.34 gallon less per 100 pounds live weight, or roughly 2.7 gallons at 800 pounds live weight. As shown in this and other investigations, the respiration rate of Brahman cattle is conspicuously lower than temperate zone breeds at high atmospheric temperature. Rectal temperature also tends to be significantly lower in so far as we have been able to secure reliable readings. Our animals for the most part have been unsympathetic and uncooperative with this scientific approach. The temperature of both the subject and the attendant doubtless rises during the struggle!

Although the presence of actively secreting sweat glands appears not to have been conclusively demonstrated, it has generally been assumed that Zebu type cattle lose more water through the skin and that water vaporization from this source plays a significant role in their higher heat tolerance. Vaporization of only 2.7 pounds of water from the skin is required to produce the same cooling as that effected by lowering the water temperatures for the Herefords in Lot 1.

Kibler and Brody (1950a) reported Holstein and Jersey cows lost comparable amounts of water of vaporization via the respiratory tract and body surface below 50°F. As environmental temperature was increased from 50° to 95°F, surface moisture losses increased more rapidly than that from respiration, reaching a maximum of 140 to 160 grams/sq. m/hr.—a level about 3 times the respiratory loss. Ragsdale, Thompson, Worstell and Brody (1950) found that Brahman cows consumed less water per 100 pounds weight below a constant temperature of 90°F. From 85°F upward the Brahmans increased water consump-

tion rapidly and exceeded with one exception the Jerseys and Holsteins. One Jersey consumed huge quantities at high temperature. The other Holsteins and Jerseys tended to decline in water consumption at high atmospheric temperature. These authors do not give the temperature of the drinking water but presumably it followed closely the ambient temperature and offered less and less inducement to consumption from the standpoint of the direct cooling effect of the water as it approached more nearly to body temperature. This might explain some of the difference in reaction of their cattle and ours but it is apparent that individual reactions may differ significantly.

Kibler and Brody (1950b) showed that the critical temperature for the Brahmans studied was 90° to 95°F compared with 70° to 80°F for Holsteins and Jerseys. They indicate that inherently lower basal metabolism and greater skin surface may be the main factors associated with heat tolerance of the Brahmans rather than greater sweating per unit of surface area. Their data show 24 hour losses from the surface of 800 to 900 pound Jerseys at 80° and above in the order of 30 pounds daily. Comparatively small increases by either increased sweating or greater area could account for a significant portion of the heat tolerance differences. More quantitative data on the physiological mechanisms of heat regulation in tropical cattle is needed. Evidently from our data higher water consumption than in British breeds is not among the reasons for their greater heat tolerance.

The philosophy that "every little bit helps" should be applied to the problems of favorably modifying effects of climatic stress on efficient livestock production. Although the number of animals in the test was limited the benefit of cooled water in terms of live weight gain is statistically significant and is supported by the data on feed consumption, respiratory rate, and general evidence of less discomfort. Feed consumption for Lot 1 (2.6 percent of body weight) is somewhat below average expectation, but the gain (average 1.46 pounds daily) is at least average for an all hay ration. Daily feed consumption of Lot 2 was 2.5 percent of body weight; Lot 3, 2.8 percent, about average expectation. The difference in feed per 100 pounds gain of 474 pounds between Lots 1 and 2, and of 837 pounds between Lots 2 and 3 emphasizes how small changes in environmental adjustment may be reflected in economy of production. Kibler, Brody, and Worstell (1949) found decreased heat production in dairy cows amounting to 30 to 40 percent when ambient temperature was increased, from 80° to 100°F. Part of this decrease was caused by declining feed consumption and milk production, but if the reaction of the cow is analogous to the rat

(Dempsey and Astwood, 1943) decline in thyroxine production may be a significant factor in observed acclimatization of cattle to hot weather. Some compensatory effects on efficiency of feed use may accrue from the metabolic adjustment as well as enhanced heat tolerance.

Cooling of drinking water in this experiment apparently added the "little bit" which tipped the balance toward the zone of thermal neutrality. The results demonstrate the significance of drinking water as a part of the heat-regulatory mechanism of cattle and emphasize the importance of finding a practical means for cooling water for animals in hot climates.

Summary

An investigation was made during the summer in the Imperial Valley of California on the effects of cooling drinking water and on the comparative water consumption of Brahman and Hereford cattle.

The mean air temperature during the test was 85.4°F; mean maximum, 100.5°F; mean minimum, 70.2. The extreme range was from 54° to 118°F.

The gains for Herefords, Lot 1, (drinking water 65°F), Herefords, Lot 2 (drinking water 88.2°F) and for Brahmans, Lot 3, (drinking water 88.2°F) were 1.46, 1.07, and 1.45 respectively. The ration was 75 percent alfalfa and 25 percent barley hay.

Daily water consumption per head averaged 15.36, 16.61, and 9.98 gallons respectively for Lots 1, 2, and 3, corresponding with 1.69, 1.89, and 1.55 gallons per 100 pounds live weight.

Data on respiration rates and on drinking habits are included. The cooled water effected an average decrease in heat load of 2800 B.t.u. per animal daily. The physiological and practical significance of the data are discussed.

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OVARIAN RESPONSE IN HEIFERS TO PROGESTERONE INJECTIONS[1]

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PREVIOUS work indicates that progesterone can be used to control heat in farm animals. It has also been shown that the response to treatment varies as the dosage of hormone is varied.

When 10 mg. of progesterone is injected daily into sheep, heat and ovulation is inhibited during the treatment interval. About 3 days after treatment is stopped estrus will occur with apparently normal ovulations. However, when the dosage level is lowered the interval from the end of treatment to heat becomes shorter and follicular growth during injections becomes greater (Dutt and Casida, 1948; O'Mary *et al.* 1950).

Daily injections of 100 mg. of progesterone will inhibit heat and ovulation in swine. If the dosage level is lowered in this species, follicular growth will also occur during treatment. On certain dosages this follicular growth will develop into a marked cystic condition which is maintained well beyond the end of the treatment period. (Ulberg, Grummer and Casida, 1951).

A preliminary study on the action of progesterone on the ovaries and estrual cycle of heifers was reported by Christian and Casida (1948). Willett (1950) studied 22 cycles in dairy heifers, where 50–100 mg. of the hormone was injected daily. Injections were started on the 14th or 15th day of the cycle and continued for 13–17 days. Estrus would occur, on the average, 5 days after the end of the injection interval, the range being 4–7 days.

It has been suggested that progesterone controls the estrual cycle by inhibiting the gonadotrophic complex, presumably LH, from acting upon the ovary. It would seem desirable to test this theory in cattle since here is a species which will allow a study of the day-to-day changes in the ovaries; this can be gotten by making daily palpations

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[3] Agents of the Bureau of Dairy Industry, U.S.D.A.

of the ovaries, *per rectum*. Also if progesterone therapy is to be used successfully in controlling follicular development and ovulation (and thereby, the estrual period), it would seem necessary to have a better understanding of the ovarian response to various dosages of the hormone.

This report deals with a study which was made to determine the effects of a range of progesterone dosages, and also the effect of varying the time and duration of treatment upon follicular development and the onset of estrus.

Materials and Methods

The effects of progesterone were studied on forty-eight estrual cycles in fourteen different heifers. These animals became available for study over a period of about 20 months. Both grade and purebred dairy heifers, ranging in age from 12–24 months were used.

The animals after purchase were brought to the experimental barns, where heat checks were made twice daily with a vasectomized bull. An animal was considered in heat only when she would stand to be served. Each was allowed to go through at least one complete estrual cycle before being put on treatment. A normal length cycle (17–24 days) had to be shown before an individual was used. Where two or more injection periods were studied in the same individual, one or more complete untreated cycles were required to intervene between the two treatments; these cycles also had to be normal in length before the animal was again put on treatment. The difficulty of obtaining animals in sufficient numbers to study one cycle per animal would have seriously decreased the number of cycles possible to study. The justification for studying more than one cycle in the same animal rested on the fact that when the estrual cycle following treatment was normal, no carry-over effect of the treatment could be detected.

Five different dosages of progesterone (PAC;[4] Lutocylin[5] and crystalline) were used (50.0; 25.0; 12.5; 6.25 and 3.125 mg. per day). The hormone was diluted in corn oil to make a concentration of 25 mg. or less per cc. The injection intervals were started at varying times in the estrual cycle. In this report the first day of heat will be considered day-1. When treatment was started on the day of heat the injections were made intravenously, all others were made subcutaneously in the shoulder region. The length of the injection intervals varied from a single injection to 28 consecutive daily injections.

[4] "Progestationally Active Concentrate" furnished by The Glidden Company, Chicago, Illinois.
[5] Furnished by Ciba Pharmaceutical Products Inc., Summit, New Jersey.

Treatments were characterized by the changes in the ovaries and the delay in the beginning of estrus. The changes in the ovaries were determined by daily palpations, *per rectum,* to make digital measurement of the follicles. Some animals were slaughtered to substantiate the palpation information. An experiment on the effects of varying the dosage of progesterone was run on 25 cycles of animals started on treatment on day-15. In this experiment five different dosage levels were studied and injections were made daily for 14 days. In the remainder of the study the time or length of treatment on a given dosage level was varied to give information on the variation in response within a dosage level.

TABLE 1. EFFECTS OF PROGESTERONE UPON SIZE OF FOLLICLE AND OCCURRENCE OF ESTRUS (BASE TREATMENT)

Dosage	Number of Cycles	Size of Follicle at End of Treatment (range and mean)	Interval-End of Treatment to Heat (range and mean)
mg.[1]		mm.	days
None	43	<10–18 (14.1)[2]	.....
50	6	<10–14 (11.3)	4–6 (5.2)
25	7	12–19 (15.5)	3–21 (6.9)[3]
12.5	3	18–28 (24.3)	2–3 (2.3)
6.25	7	12–15 (13.7)	(−7)–(−1) (−4.4)
3.125	2	14–15 (14.5)	(−5)–(−3) (−3.5)

[1] Progesterone was injected daily from day-15 of the estrual cycle through day-28.
[2] The size of the follicle at the beginning of heat when the estrus period was not under the influence of any treatment.
[3] The range and the average for this group is 3–6 and 4.5 days, respectively, when the animal which had a "quiet" ovulation during treatment is excluded.

Results

Effects of Different Dosage Levels

Heat and ovulation were suppressed during treatment in all 6 cycles studied on the 50 mg. dose (table 1). Some follicular development occurred during treatment in some animals, the maximum size of the largest follicles being 14 mm. in diameter, their average being 11.3 mm. The beginning of the post-treatment heat was 4–6 days after the last injection, with an average of 5.2 days. The follicle which ovulated from this heat was in no case the one which was estimated to be the largest at the end of treatment.

When the dosage level was decreased to 25 mg. per day (7 cycles studied), a greater amount of follicular growth occurred during treat-

ment. In all these cycles heat was prevented until after the end of the injection period. However, one animal had an ovulation without heat on day-22. Her post-treatment heat was 21 days after the end of treatment. This heat was followed by a cycle only 12 days long. The unusual length of the interval from the end of injections to heat in this animal increased the average for the group markedly. The largest follicle on this dosage level reached an estimated diameter of 19 mm. by the end of treatment. The average largest follicle for all cycles was 15.5 mm. When the animal with the "quiet" ovulation during treatment is excluded, the largest follicle at the end of the injection period was the one which usually ovulated at the post-treatment heat. This heat occurred 3–21 days after the end of injections, with an average for the 7 cycles of 6.9 days. Again if the aberrant cycle is excluded heat occurred 3–6 days after the end of injections, with an average for the 6 cycles of 4.5 days.

The estrual cycles following the post-treatment heat were considered normal in length in 5 of the 7 cycles. In the abnormal post-treatment cycles one animal came into heat and ovulated 5 days after the last injection; the life of the corpus luteum formed, however, was short and another ovulation, without heat, occurred sometime between 10–12 days later. A new corpus luteum was formed at that time and the length of the next estrual cycle was normal. The second animal was the one mentioned above which had a "quiet" ovulation during the treatment interval, where the length of the cycle following the post-treatment heat was only 12 days long.

The 3 animals receiving daily injections of 12.5 mg. each developed a large follicle during the injection interval. These follicles ranged in size from 18–28 mm. in diameter, having an average of 24.3 mm. and they seemed to be much more flaccid than follicles resulting from the 25 mg. dose. In each instance the large follicle ovulated following post-treatment estrus. No heat was observed during the injection period, but the vulva became swollen and hyperemic. There usually was a clear mucous discharge at the approximate time when the animal would have been expected in heat had she not been treated. At that time the individuals showed considerable interest in the bull and would attempt to mount the other animals, however, they would never stand for service and were therefore not considered in heat. Heat occurred, on the average, 2.3 days after the last injection. This was followed by what was considered normal estrual cycles.

Seven cycles were studied where 6.25 mg. per day were given. This dosage seemed to have only slight effect. There may have been a

tendency in some instances for heat to be delayed but heat and ovulation occurred during the injection interval in all cases. The beginning of heat, with an apparently normal ovulation, occurred from 1 to 7 days (day-21 to day-27 of the estrual cycle), before the last injection with an average of 4.4 days. The animals usually showed signs of heat for a period of 1–2 days before actually standing for service. During this period there was a mucous discharge. The injection of this amount of progesterone seemed to have no effect upon the formation or the continued function of the corpus luteum, as was indicated by the normal length of the post-treatment estrual cycle.

No marked effect could be detected on the two cycles where daily injections of 3.125 mg. of the hormone were made. Heat occurred in one heifer on day-23 and in the other on day-25. The estrual periods were considered to be normal and were followed by normal ovulations.

The Effect of Treatment When the Time and Duration Were Varied

It has been suggested that, if heat and ovulation is to be prevented in swine, the injection of progesterone must be started at least twenty-four hours before the animal is to come into heat. To study this in cattle the beginning of the injection interval was delayed until day-17 and day-19 of the cycle. Two animals were given 50 mg. daily, one started on each of the two days. In both of these instances, heat and ovulation were inhibited during the injection period, however, marked follicular growth occurred. The individual started on day-17 had a follicle estimated to be 22 mm. in diameter on day-28, the one started on day-19 and slaughtered on day-28 had a 26 mm. follicle at that time. Three animals were injected intravenously with 50 mg. daily. The first injection was made when the animal was first noticed in heat and continued until ovulation had occurred. This treatment had no effect in inhibiting ovulation or the formation of the corpus luteum. However, it may be that the hormone in oil injected directly into the blood stream actually may not be readily available to the animal.

Progesterone can be used in swine to produce a cystic condition of the follicles, which will maintain itself for at least a month after the end of the injection period. This study has shown that in dairy heifers daily injections of 12.5 mg. of the hormone is capable of inhibiting heat and ovulation but not follicular growth during a two-weeks injection period. In a further attempt to study this follicular growth the treatment interval was changed in 18 different cycles.

The first injection was made on day-15 in 14 cycles. Three of these animals came into heat and ovulated during the injection interval, two came into heat on day-30 and one on day-22. Two other animals were slaughtered on days-26 and -29. Diameters of their largest follicles were 22 and 25 mm. respectively. In 9 cycles the injection interval was prolonged until day-32 to day-42. In these individuals follicles would grow to a diameter of 20–30 mm. and then regress in size without ovulating. As the first follicle regressed a second one would replace it, either in the same ovary or in the opposite ovary. This second follicle would go through a cycle of growth and regression similar to the first and then be replaced by a third. The length of these "follicular cycles" would be about 2–3 weeks. When the injections were stopped the animals would come into heat 2–5 days later. The follicle which was growing at this time would ovulate.

This same dosage was also started on 2 animals on day-17 and 2 other animals on day-19. Follicles in the 20–30 mm. range had developed by day-28. Injections were stopped at this time. In all instances heat occurred 2 days after the last injection, following which the largest follicle ovulated. The length of the post-treatment estrual cycles were normal.

Discussion

The assumption is made that the gonadotrophic complex is made up of at least two hormones, follicle stimulating (FSH) and luteinizing hormone (LH), and that there is an interaction between these two in causing pre-ovulatory follicular growth and ovulation (Hisaw 1947). It has also been suggested from the work in swine that progesterone has a "blocking effect" upon the action of the gonadotrophic complex (perhaps LH interacting with FSH) on the ovaries.

The data in this report can be interpreted in accord with this theory. When 50 mg. of progesterone daily are given to heifers, starting before the animal's own supply of the hormone has decreased enough to allow follicular growth (day-15), the blocking effect upon the gonadotrophic complex may be complete or nearly complete. The exogenous progesterone is merely replacing the action of the decreasing endogenous supply and prevents follicles from growing beyond the pre-ovulatory swelling stage. Follicular growth up to the pre-ovulatory swelling size is assumed to be due primarily to the action of FSH.

When the beginning of the injection interval, using this same dosage, is delayed until day-17, day-19, or until the time of heat, the blocking

effect is not complete. Treatment apparently must be started before the animal's own supply of progesterone has decreased sufficiently to allow the gonadotrophic hormone to act on the ovary. This may also suggest that the blocking effect of progesterone may be due to its preventing the release of the LH from the pituitary and not so much to the blocking of the action of the hormone after it is in the blood stream.

When the daily dosage is lowered to 25.0 or 12.5 mg. the blocking effect is only partial and some of the action of the LH will be released. This LH interacting with FSH will cause a greater amount of follicular growth (up to 30 mm. in diameter) than that found in a normal untreated animal just prior to ovulation.

McNutt (1924) reported that most follicles in cattle reach a diameter of 16–19 mm. just prior to rupture but that the size reached is very irregular, for some follicles will ovulate at the time they are only 10 mm. in diameter. Forty-three follicles were checked (in individuals of this study) when the animals were first noticed in heat. These were follicles which had been formed without the influence of any treatment. The diameters of these follicles were estimated to range from 10 mm. to 18 mm. The average for the group was 14.1 mm. (table 1).

When the "lower-dosage" injections are stopped, the blocking effect is removed and the follicles will ovulate due to the increase of LH. The ability of this follicle to continue to respond to the stimulus is limited to 2–3 weeks. At the end of this period the first follicle regresses and a second follicle is stimulated to grow. If the last injection is made after a follicle begins to regress a second follicle (which has just begun to develop) will be stimulated to ovulate. This failure of ovulation also indicates a change in the sensitivity of a follicle to a given stimulus.

There is some indication that the estrual cycles following a 25 mg. treatment may be somewhat abnormal. The observation is based on too small numbers (2 out of a possible 7 cycles) to be more than suggestive of an effect. However, in gilts (Ulberg *et al.*, 1951) the greatest disruption of the estrual cycle was when 50 mg. per day was given. It was shown that when the dosage was either increased or lowered from this 50 mg. level there were no prolonged effects of the injections.

When a dosage is lowered below 12.5 mg. per day its blocking effect becomes practically *nil*. This is shown by the inability of the 6.25 mg. dosage to inhibit heat or ovulation for any appreciable length of time. This dosage may be just below the threshold needed in most individuals. The suggestion that some cycles were lengthened may indicate that the progesterone from the animal's own corpus luteum had to be

functioning at a minimum before heat or ovulation on this dosage could occur.

Summary

Five different dosages of progesterone were injected daily into 12–24-month old dairy heifers (50; 25; 12.5; 6.25 and 3.125 mg.). The beginning of the injection interval varied from day-15 of the estrual cycle to the time when the animal was first noticed in heat. The numbers of injections varied from one to twenty-eight.

The interval of time between the end of injections to beginning of heat decreased as the dosage level was decreased.

Fifty mg. daily is capable of inhibiting heat and ovulation if started before heat occurs. However, in order to inhibit most of the follicular development, the injection interval must be started earlier in the cycle (day-15).

Daily dosages of 25 or 12.5 mg. usually prevent heat and ovulation but follicles in the 20–30 mm. range will develop during treatment. Heat will occur with these follicles ovulating when the injection period is stopped. It was shown that follicles under the influence of prolonged daily injections of 12.5 mg. would regress and be replaced by another follicle at 2–3 week intervals. Dosages lower than 12.5 mg. had little, if any, effect.

The data can be interpreted in accord with the theory that progesterone inhibits the action of the gonadotrophic complex, mainly LH, from acting upon the ovary.

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THE CORRELATION BETWEEN GAINS MADE AT DIFFERENT PERIODS BY CATTLE[1]

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THE relationship of growth made by cattle during different periods of development is of general significance to the beef cattle industry. Breeding animals must often be selected at a young age, and the success of selection depends largely on the cattle retaining the characteristics for which they were selected. In the production of animals for meat, the importance of being able to predict future gains from early growth is obvious. The relationship is of special interest in the production of range feeder cattle because a question has arisen as to whether cattle that are best adapted to range conditions are most efficient in the feed lot.

Published data bearing directly on growth of beef cattle during different periods are not extensive. Hultz (1927) reported that rangy steers, which were also heavier than steers with which they were compared, gained more in the feed lot than lower-set animals. Woodward, Clark, and Cummins (1942) likewise observed that large type steers gained more in the feed lot than smaller type steers. Knox and Koger (1946) found rangy steers to gain more in the feed lot than smaller compact steers, and suggested that the difference in gain was likely due to size rather than type. Lush (1932) and Stanley and McCall (1945) reported a positive correlation between rate of feed-lot gain and skeletal size.

These reports indicate that growth prior to feeding age is correlated positively with gain in the feed lot. Contrary to this, Black *et al.* (1938) found height at withers and body length to be negatively correlated with feed-lot gain. Width was positively associated with gain. These data were taken from beef, dual purpose, and dairy animals, and breed differences may have entered into the results.

Source of Material

The records used in this study were from animals produced in the herd of grade Hereford cattle on the semidesert experimental range of

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the New Mexico College of A. & M. A. Each fall for 13 years, 30 to 40 weaning steer calves were kept and run on the range until the following fall, when they were taken to the feeding pens at the College, fed for 168 days, and finished at a grade of good to choice. Records on these steers are part of the material for this study. The second group of growth records were from 20 to 30 heifer calves that were kept each year from 1935 to 1945 as replacements in the range breeding herd. The third series of records show the growth curves of calves produced since 1945 in a type-size experiment.

The calves were dropped from March 1 to June 1, with a majority being born in April. Each year, in October or early November, the calves were all weaned together, weighed, and graded. Except for two groups of calves from the type-size experiment that were taken directly to the feeding pens at weaning, the calves were fed a small amount of cake for a short time following weaning, and the heifers separated from the steers. The heifers were run on the range all in one group until they first calved at 3 years of age. Thereafter they were scattered among different pastures on a relatively uniform range. The steers fed as long yearlings were also run in one group until they were moved to the feeding pens.

Results

Steers Fed as Long Yearlings. The lighter weight steer calves at weaning were held over to be fed as long yearlings and thus were not strictly a random group. They were light, however, mainly because they were younger than average, and except for age, they were thought to be a cross section of the steers produced.

Weight gains were recorded for the following periods: From conception to weaning, or weaning weight; from weaning until the following fall, referred to as yearling gain; from conception to long yearling age; and the feeding period of 168 days.

Weaning weight was expressed in three different ways, actual weight at weaning with no correction for age of calf or age of dam, weaning weight corrected for age of calf (Koger and Knox, 1945), and weaning weight corrected for age of calf and for age of dam (Knox and Koger, 1945). The uncorrected weight was used to determine which calves, regardless of age, made the largest yearling gains, and to determine the value of the corrections in increasing the correlation with subsequent gains. Weaning weight standardized for age of calf was used as a measure of the rate of gain from conception to weaning. Standardization for age of dam was a partial correction for differences in condition

since age of dam markedly affects milk production, and consequently, condition and growth rate of calf (Knox and Koger, 1945).

In the feed lot, the steers were divided into four groups and fed different rations, making necessary a slight correction for feed-lot gain. This correction was made by adding or subtracting the difference between lot mean and average of all groups to the gain of each individual in the lot.

The records were tabulated by sire groups for each year. Sums of squares and products within sire groups were calculated and pooled and

TABLE 1. MEAN VALUES OF TRAITS AND CORRELATION OF GAINS MADE AT DIFFERENT PERIODS BY STEERS

Weights and Gains	Mean lbs.	Correlation Coefficient[1] with Yearling Gain	Correlation Coefficient[1] with Feed-lot Gain
Weaning Weight, Uncorrected	432	—.16	.04
		—.14	.01
Weaning Weight, Standardized to 205 days of age	437	—.10	.17
		—.08	.19
Weaning Weight, Standardized for age of calf and computed to mature age of dam	451	.04	.28
		.09	.27
Yearling Gain	314	...	.17
			.43
Long Yearling Weight	746	...	.25
			.39
Feed Lot Gain	369	...	...

[1] The coefficients shown first were calculated within sire groups and years. df=354. Those shown on lower line are inter-sire relationships calculated from the averages of sire groups within years. df=54.

the various correlations determined. The inter-sire relationships were calculated from the average of sire groups within years. There were 424 steers sired by 68 bulls in 13 years.

Within sire groups (table 1), uncorrected weaning weight showed a significant negative correlation of 0.160 with yearling gain, and showed no significant relationship to feed-lot gain. Correlation of weaning weight corrected for age of calf with yearling gain was negative and not significant, while with feed-lot gain it was significant and positive. The additional correction for age of dam resulted in a higher correlation of weaning weight with feed-lot gain and a non-significant positive

correlation with yearling gain. Yearling gain and long yearling weight both showed significant positive correlations with feed-lot gain.

The inter-sire correlations, shown also in table 1, were similar to those obtained within sire groups. This would be expected since the differences in corrected weaning weight were due largely to variation in the suckling ability of dams rather than to the influence of sires (Koger and Knox, 1947; Koger, 1948). The inter-sire correlations of yearling gain and yearling weight with feed-lot gain were higher than those calculated within sire groups. Apparently, differences in sire groups are more indicative of inherent differences in growth potential than variations in individual weights where differences in condition are more pronounced.

Data from Heifers. Of the heifers born between 1935 and 1945 and kept as replacements for the breeding herd, 155 reached maturity and reproduced regularly. The records from these heifers were used in this study. The heifers graded higher, were 6 days older, and had a corrected weaning weight 9 pounds greater than the average of all heifers in the herd. There is no apparent reason, however, why this amount of selection should bias the relationships shown in the data.

The records used were uncorrected weaning weight, weaning weight corrected for age of calf, weaning weight corrected for age of calf and age of dam, gain made during the year following weaning (referred to as yearling gain), weaning weight plus yearling gain (long yearling weight), 2-year-old gain, 3-year-old weight, gain from 3 years to maturity, and mature weight taken at 7 years of age. The records were classified by year and sire, and correlations between the various measurements were determined within sire groups and years. Inter-sire relationships were determined from sire groups within years. A summary of the data is shown in table 2.

As in the case of steers, uncorrected weaning weight within sire groups was negatively correlated with yearling gain, and showed no significant relationship with gain made after 2 years of age. Weaning weight adjusted for age of calf was significantly and positively correlated with 2-year-old gain, but its relationship with yearling gain was still negative. With the additional correction for age of dam, weaning weight showed significant positive correlation with subsequent gain to 3 years of age. Weight increase after 3 years showed no significant relationship to growth made at earlier periods. This is to be expected, since, under range conditions, gains after calving seem more

strongly influenced by suckling ability and other factors than by growth potential.

The coefficients calculated from the average of sire groups (table 2) were similar to those calculated within sire groups except that the correlations were higher and were positive in more comparisons.

TABLE 2. MEAN VALUES OF TRAITS AND CORRELATION BETWEEN GAINS MADE AT DIFFERENT PERIODS BY RANGE HEIFERS

Weights and Gains	Mean lbs.	Correlation Coefficient[1] with Yearling gain	Gain from long yearling to 3 years	Gain from 3 yrs. to maturity
Weaning Weight, Uncorrected	426	—.15	.07	—.01
		—.13	.30	.02
Weaning Weight, Standardized to 205 days of age	419	—.13	.21	.01
		—.02	.26	.00
Weaning Weight, Standardized to 205 days of age and computed to mature age of dam	441	—.11	.27	.03
		.07	.35	.07
Yearling Gain	204	...	.06	.04
			.12	.05
Long Yearling Weight	630	...	.13	—.03
			.24	.01
Gain from Long Yearling to 3 Yrs.	268	...	...	.10
				.13
Three-Year-Old Weight	898	...	...	—.02
				.03
Gain from 3 Yrs. to Maturity	99	...	...	...

[1] The coefficients shown first were calculated within sire groups and years. df.=107. Those shown on the lower line are inter-sire relationships calculated from the averages of sire groups within years. df.=37.

Comparison of Growth Made by Different Sized Calves in Similar Condition

From the results obtained above it appeared that the variance in weight of calves within a group was due partly to inherent differences in growth potential which were apparent throughout the growth period and partly to differences in condition and growth for which there was an inverse compensation in later growth. Thus, it appeared desirable to study the regularity of difference in growth rate when nutritional level and condition were similar. Data from a type-size study provided

records on two groups of calves that showed variation in growth rate but from slaughter data (unpublished) and visual estimates of conditions were known to be in similar conditions throughout their growth period.

The animals from which the growth records were taken were the calves from one group of cows which were selected for heavy weight and another on the basis of type with no consideration given to weight. The two groups of cows differed in mature weight by approximately 150 pounds. The heifer calves were handled in the same manner as the other replacement heifers. The steer calves from two calf crops were run on the range until they were long yearlings before they were moved to the feed lot. Steers from two other calf crops were taken to the feeding pens directly on weaning and fed for a period of 196 days.

The growth data from these groups of animals are shown in figure 1. Weight differences at weaning time became greater as the animals increased in size on the range or in the feed lot. This indicates, as did the data on the groups above that the relative growth rate of young animals of the same age and conditions is indicative of future growth potential.

Discussion

There are two opposing influences on the relationship of gains made at different periods of growth in animals. In groups where age of maturity is relatively uniform, individuals that reach a large mature size must necessarily have a high growth rate throughout all, or most of the growth period. Conversely, the smaller individuals of a population do not at any period gain extremely rapidly, or else they would not end up in the small group. This influence tends to result in growth at different periods showing an automatic positive correlation. On the other hand, in animals where mature size is similar, the greater the amount of growth which is realized at a given age, the less the amount which is left to attain later and vice versa. This would tend toward a negative relationship between growth made at different periods.

Thus, a positive relationship between growth at different periods would be most in evidence where the environment to which the different animals are subjected is held uniform. A negative relationship would be expected when environmental variations are experienced during one period and later removed, allowing a compensation for over or under growth during the first period.

The net result is a balance between the two influences, and it is determined largely by the conditions under which growth is made. In this study, conditions were such that, in general, growth at different periods showed a positive relationship. Since correction for age of dam at weaning made the relation with the subsequent growth more positive, it appears that if corrections could have been made for other differences in condition, the relationship would have been more strongly positive. This thesis is supported by the fact that selection for size has been highly successful (figure 1 and unpublished data).

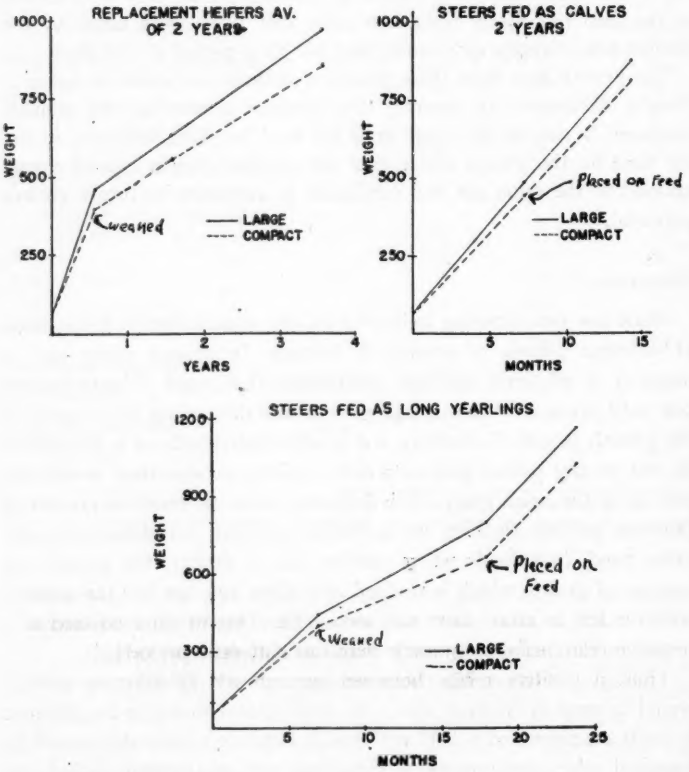

Figure 1. Growth curves of offspring from one group of cows selected for heavy weight and from another group selected for compactness, average of two calf crops.

It is encouraging, from a practical viewpoint, that adaptability to range conditions, as measured by growth, showed a positive correlation with feed-lot gain. This study indicates that better adaptability to the range will be associated with improved feeding qualities.

Summary

Records from beef cattle produced on the experimental ranch of the New Mexico Agricultural Experiment Station were analyzed to determine the relationship of growth rates at different periods of development.

The growth rate of steers on the range was positively correlated with their gain in the feed lot. In general, growth on the range at different periods was also positively correlated. Growth during periods in which the animals were subject to variable external influences, such as variable milk supply from dams, showed a low, or in some instances, a negative relationship to growth at other periods.

It was concluded that, when environment is constant for different animals, there is a positive relationship between gains made at different periods. This relationship can be obscured or even reversed by variable conditions. There was a positive relationship between performance on the range and in the feed lot.

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SIZE OF CALVES AT WEANING AS A PERMANENT CHARACTERISTIC OF RANGE HEREFORD COWS[1]

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THE extent to which the weaning weight of the calf is a permanent characteristic of its dam is an important part of the problem of selecting cows to improve their productivity. Repeatability of weaning weight is needed to compare fairly the producing abilities of cows which have had different numbers of calves. The younger the cow when her producing ability can be known accurately, the more efficient the selection can be. The weaning weight of the calf is a useful measure of a cow's yearly production, since this observation is taken at the end of the period over which she exerts maximum influence on the growth of the calf. Many range calves are sold at or shortly after weaning and their weight determines to a large extent the amount the owner receives.

Koger and Knox (1947) measured the repeatability of weaning weight of calves from range Hereford cows by computing the correlation between consecutive records of the same cow. The average correlation between the weights of consecutive calves by the same cow was 0.49. Gregory *et al.* (1950), studying beef cattle weaning weights, obtained values ranging from 0.37 to 0.50 for the correlation between various calves from the same cow. Sidwell and Grandstaff (1949) obtained a value of 0.22 for the repeatability of weaning weight of lambs by Navajo ewes. Lush and Molln (1942) investigated the extent to which the weaning weight of litters was a permanent characteristic of sows and found a value of 0.13.

Data and Methods

Source of data

The animals used in this study were purebred or high grade Herefords from Line 1 at the U. S. Range Livestock Experiment Station, Miles City, Montana. The data are the weaning weights of 745 calves

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from 180 cows having two or more calves during the period 1938–1948. All calves were born on the range during April and May. The calves were weaned about October 20. All cows were kept together on the range, except during the breeding season. The breeding season was limited to six weeks, so that all calves each year were produced within a period of approximately eight weeks.

Analysis of data

The least squares method of estimation for multiple classifications with disproportionate frequencies was used in the analysis of the data (Yates, 1934; Hazel, 1946). Each observation was assumed to be the sum of the influences or effects of the identifiable variables as follows: Weaning weight=general mean+year+age of dam+cow+sex+age of calf at weaning+inbreeding of the dam+inbreeding of the calf +effects peculiar to each calf. This linear combination of effects is referred to hereafter as the mathematical model.

The general mean in the mathematical model has as its basis the fundamental likeness of the observations, due to the fact that all calves belong to the same genus, species, and breed, and as such have certain anatomical and physiological properties in common; also the calves studied here had common elements in their ecology. Superimposed on this common portion are other influences affecting weaning weight which are not alike for all of the calves. *Year* effects are caused mainly by year-to-year fluctuations in the temperature and precipitation. These fluctuations affect markedly the forage available on the range, the comfort of the animals, and the amount of disease or parasites present in any year. Changes in management practices will also be reflected in the year effects. *Age of dam* affects the weaning weight of calves presumably through changes in the udder development and milking ability to withstand the rigors of range conditions. For measuring the effects of *sex* differences in this study the calves were classified into three groups: heifers, bulls, and steers. The *age of calf at weaning* is a cause of some variation in weaning weight, because the calves were born during a two-month interval but were weaned at the same time. The *inbreeding of the dam* and *inbreeding of the calf* were other sources considered because most of the published reports indicate that inbreeding has a detrimental effect upon functional characters. As yet, almost nothing is known definitely about how inbreeding influences weaning weight in beef cattle. The *errors* or *effects peculiar to each individual* calf include all the other sources of variation. These include

genetic differences between calves in their growing abilities (so far as those are not included in the cow effects), accidents and environmental details peculiar to the individual, and all other unanalysed sources of variance. Any discrepancies between the mathematical model and the actual way the effects of the variables are combined would be included here.

Difference between *cows* is the most important aspect of this study, because this is the main element in repeatability. Cow differences refer here to the differences in the mean weaning weights of calves produced by different cows, the records having been adjusted to a comparable basis for the other factors. Cow differences include both genetic and permanent environmental differences. The cow's genotype determines (along with Mendelian segregation) the genes she transmits to each calf. It also affects her own inherent milking ability and her competence under range conditions. Permanent environmental influences are the non-hereditary factors affecting a cow's producing ability which remain relatively constant throughout her life. Factors such as stunting when young, permanent injury to the udder, and crippling are examples.

Applying the mathematical model to the data gives a system of 205 simultaneous equations which must be solved to obtain estimates of the different effects and to complete the analysis.

Results

Estimates of effects and analysis of variance

Due to a linear dependency between years and age of dam, a unique solution for all the effects was not possible. The dependency arises because the calving pattern for each cow fixes both the years she appears as a dam and the ages at which she is a dam. Biologically, there must be a perfect correspondence between the two classifications for each cow. Statistically, this amounts to there being one less degree of freedom associated with the set of equations than is apparent. Consequently, one additional restriction to those normally applied for solving the least squares equations had to be made to obtain a numerical solution. This was done by assuming a value for one of the effects in the dependent set and then solving the remaining equations. Although the size of the assumed value affects the numerical value of members of the dependent set, the variances are the same for all solutions. Unique estimates were obtained for the sex differences and for the regressions. The sex effects obtained are expressed as deviations from

TABLE 1. ESTIMATES OF EFFECTS

Item		Estimate of Effect (Pounds)
Sex	Bull—deviation from the mean[1]	25.0
	Heifer—deviation from the mean[1]	—18.8
	Steer—deviation from the mean[1]	— 6.3
Regression of weight on age at weaning (per day)[2]		2.27
Regression of weight on inbreeding of dam (per one percent)[3]		—2.54
Regression of weight on inbreeding of calf (per one percent)[4]		—0.48

[1] Mean weight of calves 393 lbs.
[2] Mean age of calves 176 days
[3] Mean inbreeding of dams 5.9%
[4] Mean inbreeding of calves 12.4%

a mean while the regressions represent the average change in weight of calf for each unit of change in each of the three independent variables (table 1).

A summary of the analysis of variance is given in table 2. The mean square for the variance among cows is highly significant, the F value being 5.5. The method of analysis permits isolating the variance due to each source separately, but only the mean square among cows is shown here, as it is the most pertinent to this study.

Repeatability

The degree of likeness of repeated records has been termed the repeatability of the characteristic (Lush and Molln, 1942). It is the

TABLE 2. ANALYSIS OF VARIANCE

Source	d/f	Sum of Squares	Mean Square
Reduction due to fitting all constants	200	2,095,906	
Reduction due to fitting all but cows	21	1,391,596	
Among cows	179	704,310	3,935
Error	544	391,968	.721
Total	744	2,487,874	

correlation between two random records of the same cow. In terms of variance components, repeatability is $\frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2}$ and is the intra-class correlation among records by the same cow. The σ_c^2 is the variance of cow effects on weaning weight and σ_e^2 is the error or variance among calves from the same cow. The estimate of σ_e^2 is obtained directly from the analysis of variance (721 in table 2). The expected value of the mean square among cows is $\sigma_e^2 + k\sigma_c^2$. The k is the effective number of calves in each cow subclass. In this study k *was* 4.13.

Taking the mean squares from table 2 and using 4.13 for k, we obtain the following estimate for repeatability.

$$\sigma_c^2 = \frac{3935-721}{4.13} = 778$$

$$\text{Repeatability} = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2} = \frac{778}{1499} = .52$$

The approximate 95 per cent confidence interval was computed according to Fisher (1950, p. 218 and 225). The lower limit is .44 and the upper limit is .60.

Discussion

The linear dependency mentioned previously prevented measuring independently the effects of cows, years, and age of dam, although the variances were not affected. The estimates of the sex differences, the linear regressions of weight on age of calf, and on inbreeding of calf are, however, independent and unbiased estimates of these effects.

Bulls and steers were 44 and 13 pounds heavier, respectively, than heifers. Previous investigators have noted a significant difference in the weaning weights of male and female calves. The large difference of 31 pounds between steers and bulls may be due to the effects of the selection which was practised in deciding whether or not to castrate each male calf. More of the small than of the large bulls would have been steered, unless extreme emphasis were placed on pedigree and family performance when deciding which males were to be kept as bulls. Even then the larger animals would have been selected if other things had been nearly equal. Possibly some of this 31 pound difference may have resulted from the steers not having had sufficient time to overcome the setback caused by castration. The 31 pound difference is not by any means to be interpreted wholly as a physiological result of castration. If we take the weighted average of bull and steer differences found in this study, we obtain an average difference of 23 pounds between males and females. This is in agreement with the average

difference of 22 pounds between males and females found by Knapp *et al.* (1942) on other data.

The regression of weight on age of calf at weaning found in this study (2.27 lbs. per day) is higher than that found by other workers studying range cattle, although it does not seem unreasonably large for calves averaging 393 pounds at 176 days. Several reasons may account for this large value. The data are from Line 1 cattle which are the most rapid-gaining cattle at the Miles City station. Another reason for the high regression coefficient might be that the nutrient value of the forage on this range does not decline as greatly about weaning time in the fall as is true on some ranges. Some data show a lower regression of weight on age of calf about weaning time in the fall as compared to that found at an earlier period, presumably when range conditions are better. The regression obtained from these data depends upon the variation in age and weight of the calves at the date weaned. The procedure used gives the best fitting straight line for weight of calf on age with the effects of other variables held constant statistically. Implicit in this method is the assumption that calves born early or late in the season grow at the same rate at the same ages. If calves born early in the season actually grow at a slightly faster rate than those born later, the regression as calculated here is larger than the actual average growth rates of the calves over this period.

The regression of weight of calf on inbreeding of the dam was —2.54 lbs. for each percent of inbreeding. This regression could be estimated only when constants for cows were not fitted. For this regression to measure only the effect of the inbreeding of the cow, the effect which the inbreeding of the cow has on weaning weight must be uncorrelated with the other elements of a cow's producing ability, such as milking capacity and competence on the range.

The effects of inbreeding of the calf on weaning weight found in this study (—0.48 lb. for each per cent of inbreeding) is in accord with the findings of several other workers.

The repeatability of such characters as birth weight or weaning weight must be interpreted somewhat differently from the repeatability of such things as milk or wool production. The weaning weight of an animal occurs only once in the individual's lifetime and is repeatable only when considered as a characteristic of the dam. A cow influences the weaning weight of a calf both by the genes she transmits to the calf and by the maternal environment she provides the calf from conception until weaning. The distinction must be kept clear when relating the estimates of repeatability to heritability. Repeatability, as considered in this study, sets an upper limit to the heritability of the

maternal influence on weaning weights. This is not equivalent to the heritability of weaning weights considered as a characteristic of the calf, such as the 0.28 obtained from paternal half-sib correlation and sire off-spring regression methods by Knapp and Clark (1950).

The repeatability calculated from these data is based on differences between cows which had calves during a 10-year interval. A cow's effect is here expressed as a deviation from the average of all cows having calves during this 10-year period. If instead, we express a cow's effect on the weaning weight of her calf as the average of all cows born in the same year plus her deviation from that average as suggested by Henderson (1949), then the variance among cows as found in this study has in it some of the variance among the means of birth-year-groups, in addition to the variance among cows born in the same year. The remainder of the variance between birth-year-groups goes out with the other classifications, such as year and age of dam, the amount being proportional to the square of the multiple correlation between the means of the birth-year-groups and the other classifications. If little or no change occurred in the mean abilities of the cows during the period covered by this study, the contribution to the variance among cows from the variation in the means of the birth-year-groups would be small. The repeatability of weaning weight as obtained in this study may then be too high for comparing cows born in the same year and too small for comparing cows born about ten years apart.

Lush (1947) states the most probable producing ability of a cow $=$ herd average $+ \frac{nr}{1 + (n-1)r}$ (cow's average minus the herd average), where n equals the number of records making up the cow's average record and r is the repeatability coefficient. This equation may be used where records have been adjusted for environmental factors such as those considered in this study. The application of the repeatability obtained is limited to those situations where cattle are handled under similar conditions and the data are corrected for the same sources of variation, exclusive of cows, as in this study.

The comparatively high repeatability of weaning weights indicates that selection for cows which will wean heavy calves can be practiced early in their producing life. A more detailed study, including estimates of heritability, would be required to determine how much of any improvement accomplished would be genetic.

Summary

The extent to which the weaning weight of her calf is a permanent characteristic of a range Hereford cow was studied, using the weaning

weights of all the 745 calves from the 180 Line 1 cows which had calved at the Miles City Station at least twice during the period 1938–1948.

The records were adjusted for the following factors thought to influence the weaning weight of calves: Year in which the record was made, age of dam, sex of calf, age at weaning, inbreeding of dam, and inbreeding of calf.

The average uncorrected weight of all calves was 393 lbs. The standard deviation of adjusted weights was 27 pounds. The average age at weaning was 176 days. The average inbreeding of all calves was 12.4%. The average inbreeding of all dams was 5.9%.

Differences between cows accounted for 52 percent of the variance in the calves corrected weaning weights. Therefore, the extent to which the weaning weight of calves is a permanent characteristic of range Hereford cows, as determined from this study, is 0.52. The repeatability of 0.52 is based on differences between cows which made their records during a 10-year interval and may, therefore, be slightly high for comparing cows born in the same year.

This repeatability is high enough to permit reasonably accurate selection of cows for high life-time production on the basis of the first calf weaned.

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NEWS AND NOTES

The forty-third annual meeting of the American Society of Animal Production will be held in the Hotel Sherman in Chicago, Illinois on November 23 and 24, 1951 with a dinner for the honored guest on November 26.

In accordance with Article 2, Section 3 of the constitution of the American Society of Animal Production (Journal of Animal Science 2:95–99, 1943) the Executive Committee has adopted, after consultation with the Editorial Board, the following schedule of dues, subscription rates and reprint and publication charges, to offset costs to the Society, particularly for publishing the Journal of Animal Science. Changes in reprint and publication rates shall be effective for the November, 1951, issue of the Journal, but changes in dues and subscription rates shall become effective Jan. 1, 1952. The increasing numbers of manuscripts and increasing costs of printing have forced the Executive Committee to require of authors that they purchase 100 reprints of their papers at a rate that will pay a share of the printing costs of their papers and to raise membership fees and subscription rates. It is believed that institutions that support research in animal science should assume the responsibility of sharing in the costs of printing the results of such research. Hence, the following action is being taken by the Executive Committee:

Membership Fee—$5.00 per annum
Subscription rate (non-member)—$6.00 per annum
Required reprint purchase (100 copies without covers) at $6.50 per page of article
Additional optional reprints without covers at $0.75 per page of article per 100 copies
Additional charges:
Cuts (beyond one page per paper) $10.00 per page
Tables (beyond two pages per paper) $8.00 per page
Reprint covers—100 at $8.00. Additional, 3 cents each.

Authors will be asked to indicate when their manuscripts are accepted the name and address of the institution, agency, or individual who will assume responsibility for the reprint and additional charges.

The Second International Congress of Physiology and Pathology of Animal Reproduction and of Artificial Insemination will be held in Copenhagen, Denmark, from July 7th to 10th, 1952, with the following program:

1. The physiology of reproduction
2. The pathology of reproduction
3. Artificial insemination of domestic animals.

The Standing Committee has nominated John Hammond, School of Agriculture, Cambridge, as president, Nils Lagerlof, Stockholm, as vice-president, and Ed. Sørensen, Copenhagen, as secretary-general. The Congress is sponsored by the Danish Ministry of Agriculture and will be organized by the Royal Veterinary and Agricultural College, Copenhagen, the Danish Federation of Cattle Breeding Societies for A.I., and the Society of Danish Veterinary Surgeons. All particulars are obtainable from the Secretary-general, Ed. Sørensen, The Royal Veterinary and Agricultural College, Bulowsvej 13, V. Denmark.

The Vitamin B Complex, by F. A. Robinson, published by John Wiley and Sons, Inc., New York, in 1951, is a critical review of published material. Extensive literature references are given. It covers methods of assay, methods of synthesis and nature of deficiencies. The importance of this complex in the metabolism of all living organisms is emphasized.

The Biochemistry of Fish is the report of a Biochemical Society Symposium edited by R. T. Williams and published by the Cambridge University Press in 1951. Topics of interest to nutritionists have to do with the proteins, fats, nitrogenous extractives, carotenoids and bile salts of fish.

Man and the Animal World by Bernal R. Weimer was published by John Wiley and Sons Inc. in May 1951. The animal groups are dealt with from the standpoint of relationship and universality of life functions. Stress is placed on the importance of the various groups to man in terms of economics, health and disease. Emphasis is placed on scientific method.

William M. Regan retired from active duty July 1, 1951 after having directed the dairy production work at the University of California for 29 years.

Clifton D. Lowe retired from active duty as extension Animal husbandman in the U. S. Department of Agriculture on June 30, 1951. He had served nearly 28 years in that position.

The National Research Council advises that the authoritative booklets, Recommended Nutrient Allowances for Swine; Recommended Nutritive Allowances for Dairy Cattle; Recommended Nutrient Allowances for Sheep; and Recommended Nutrient Allowances for Horses, being separate reports of their Committee on Nutrition, are available from their office, 2101 Constitution Ave., N.W., Washington 25, D. C.

Frank J. Welch, Dean of Agriculture and Director of the Experiment Station, Mississippi State College for several years, became Dean of Agriculture and Home Economics, Director of the Agricultural Experiment Station and Director of the Cooperative Extension Service at the University of Kentucky on July 1, succeeding Thomas P. Cooper, who assumes emeritus status.

Rex Beresford, Iowa State Agricultural Extension Service; Robert E. Hutton, U. S. Range Livestock Experiment Station; Julius E. Nordby, U. S. Western Regional Sheep Breeding Laboratory, and Clifton D. Lowe, Extension Service, U.S.D.A., were the animal husbandry workers, receiving Superior Service Awards at the hands of Secretary of Agriculture Brannan, at the annual ceremony staged on the Washington Monument Grounds in Washington, D. C. on May 15, 1951.

Phil S. Eckert, an agricultural economist, was made Dean of Agriculture and Director of the Experiment Station, University of Arizona, on July 1, succeeding Paul S. Burgess, who voluntarily retired from administrative responsibilities.

E. W. Janike, former extension animal husbandman and more recently State 4-H Club leader at the University of Nebraska, has been made Associate Director of Extension in the same institution.

Louis B. Howard, head of food technology, University of Illinois, has been named Associate Director of the Illinois Agricultural Experiment Station, to succeed W. E. Carroll, who retires from that position September 1, 1951.

Elmer H. Hughes, head of the animal husbandry department, University of California, died suddenly on April 16, 1951.

John I. Hill, Vice President, University of Wyoming, and honor guest in 1949 of the "Society", died suddenly on March 10, 1951.

Horse Husbandry, is the title of a new book of 336 pages by M. E. Ensminger of the State College of Washington, published by The Interstate of Danville, Illinois.

Karl Rasmussen, a native of Illinois and holding degrees from the University of Manitoba, University of California, and Iowa State College, is now head animal husbandman for the Dominion Experimental Farms, throughout Canada.

Wise Burroughs, formerly with the Ohio Agricultural Experiment Station has transferred to Iowa where he is associate professor of Animal Husbandry with the Iowa State College.

Ray H. Means, formerly an animal husbandman on the Mississippi State College staff is now in charge of livestock production for the Delta and Pine Land Co., at Scott, Mississippi, which operates a large farming enterprise.

O. Burr Ross, formerly of the animal nutrition staff, Oklahoma A & M College, resigned on March 1, to take charge of the livestock and poultry production program of the Gooch Feed Mill Company of Salina, Kansas.

H. H. Levick, of Mississippi State College, has been promoted from the position of head of the animal husbandry department to Associate Director of the Agricultural Experiment Station.

DeWitt C. Wing, of Breeders' Gazette, Rural New-Yorker, and farm press fame generally, and who spent more than 10 years in U.S.D.A. information work passed away at Normal, Illinois, on March 31, 1951.

Kenneth F. Warner, extension meat specialist, U.S.D.A., has transferred to the Extension Education and Training Division of the Office of Foreign Agricultural Relations for an indefinite period of service.

A. T. Edinger, long-time worker in the field of meat with Federal agencies, has been retired for some months from U.S.D.A. and is now living in Chillicothe, Ohio.

Earl B. Krantz, of the U. S. Morgan Horse Farm, Middlebury, Vermont, and S. R. Speelman, of the Agricultural Research Center, Beltsville, Md., recently retired from official duties with the U.S.D.A.

W. T. Anderson, Purdue University, and Charles E. Bell, Jr., University of Georgia, both extension specialists in animal husbandry for their respective institutions were guests of Swift and Company on an educational tour this spring.

W. B. Connell, veteran extension animal husbandman for Pennsylvania State College, retired from public service on June 30, 1951.

Boyd T. Whittle, extension specialist in animal husbandry, University of Maryland, has been recalled to active duty with the U. S. Army. During Mr. Whittle's absence his duties are being assumed by Martin E. Gannon.

Richard O. Smith, assistant professor of animal husbandry, South Dakota State College, became manager of By-The-Way Ranch in Nebraska on May 1. Dr. Smith is a 1940 graduate of the Ohio State University and spent 52 months in the Armed Services during World War II.

M. H. McDonald, extension animal husbandman at North Dakota Agricultural College, recently resigned to become a fieldman for the American Hereford Association in the California, Arizona, Nevada and Utah territory.

Ned Tyler, of the animal husbandry department, North Carolina State College, has accepted the position of field representative for The Breeder-Stockmen of Warrenton, Virginia.

Paul M. Grinde, extension animal husbandman, New Jersey State Agricultural College, has resigned to accept the position of manager of a prominent cattle breeding farm in New Jersey.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should contain a clear statement of the purpose of the investigation and its relation to other work in the same field, but long reviews should be avoided. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results, or their relation to some problem of the livestock industry. Each paper should end with a brief summary outlining the points proved in the investigation and their application, or their relation to a livestock problem.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. The author or his institution, except in the case of invited review papers, will be required to purchase 100 reprints of his paper at a rate that will pay a share of the printing costs. There will also be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and also at that time to order any additional reprints he may want.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts". *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest concerning the members, and other items of interest to the membership. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. L. E. Casida, Department of Genetics, University of Wisconsin, Madison, Wisconsin, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. J. I. Miller, Department of Animal Husbandry, Cornell University, Ithaca, N. Y., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.